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Effect of Formaldehyde upon the Sensitizing Property of Monilia.

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In a previous work[1] we were able to show that regularly constant sensitization of guinea pigs could be produced through the inoculations of heat-killed agar cultures of different moniliae. A comparative study of the sensitizing capacity of the *Monilia pinoyi* and *Monilia psilosis* demonstrated that while the former fungus could sensitize guinea pigs even after a single injection of a moderate dose of the culture, it was necessary to use repeated injections of the latter in order to obtain a successful sensitization of the animals. In view of the close serological and morphological relationship between these two species such a difference in the sensitizing capacity was difficult to explain.

On the basis of the above study the suggestion occurred to us that the sensitizing capacity of different organisms such as moniliae may represent an independent function of the fungus protein. If such be the case, it would seem reasonable to expect that this function of the fungus may be diminished or completely destroyed through the use of a suitable procedure. It is known that the addition of formaldehyde to bacterial products, such as toxins, or to bacterial vaccines brings about a considerable diminution or destruction of the

[1] Kurotchkin, T. J., and Lim, C. E., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 223.

toxic principle. Of especial significance is the fact that within certain limits, the removal of the toxic principle by formaldehyde is not associated with the decrease or disappearance of the antigenic property of bacterial toxins or vaccines.

Considering this peculiar action of formaldehyde it seemed of interest to test its effect upon the sensitizing power of the monilia cultures. For our experiment, *Monilia pinoyi*, the agar culture of which possessed a high sensitizing power, was selected. The fungus was grown for 48 hours at 37°C. and then its growth was washed off with normal saline. A sufficient quantity of fungous suspension was thus prepared. The bulk of the suspension was then divided into 6 equal parts. One part was exposed to 75°C. of heat in water bath for 30 minutes and then incubated for 20 days at 37°C. The remaining 5 parts of the monilia suspension received the addition of formaldehyde in concentrations of 1:5, 1:10, 1:50, 1:100, and 1:500 respectively. These were left in the incubator for 10 days and then each suspension was centrifuged, washed with normal saline and resuspended in the same volume of saline as used for the original preparation. With each suspension a series of young guinea pigs of approximately equal weight was sensitized by giving 3 intraperitoneal injections, each consisting of 1 cc. of the respective suspension. One control series of guinea pigs was sensitized by injections of heat-killed monilia suspension prepared from a 48-hour agar growth. Another control series was sensitized by injections of heat-killed monilia suspension kept in incubator for 20 days before use.

TABLE I.

Showing sensitization of guinea pigs with formalin or heat-killed cultures of *Monilia pinoyi*.

Series	No. of animals	Kind of vaccine used for sensitization Monilia culture treated with formaldehyde	Result
1	3	1:5	No symptoms
	2		Slight shock
	2		Fatal shock
2	9	1:10	No symptoms
	1		Slight shock
3	7	1:50	No symptoms
	3		Moderate shock
4	5	1:100	No symptoms
	1		Slight shock
5	4	1:500	No symptoms
	3		Fatal shock
6	6	Heat-killed and incubated 20 days at 37°C.	No symptoms
	1		Moderate shock
7	2	Heat-killed freshly prepared	" "
	10		Fatal shock

Two weeks after the last injection all animals were tested for hypersensitivity by injecting one or 2 mg. of the specific carbohydrate obtained from *Monilia pinoyi*. The result of the experiment is given in Table I. It will be seen that freshly prepared heat-killed culture of the *Monilia pinoyi* produced sensitization of guinea pigs in all instances. In contrast to this, the heat-killed culture which was kept in the incubator for 20 days before use showed a definitely lower sensitizing capacity, since out of 7 animals only one developed moderate shock. Formalin-treated suspensions gave somewhat variable results. The addition of formaldehyde in a concentration of 1:5 produced no appreciable effect upon the sensitizing power of the monilia culture. With the next concentration of 1:10 a marked diminution of this power occurred. Concentrations of 1:50 and 1:100 have also brought about a definite decrease of the sensitizing power. The result with a concentration of 1:500 was inconclusive in view of the fact that almost 50% of animals were thrown into anaphylactic shock.

In the interpretation of these results it should be borne in mind that the individual response of guinea pigs to the anaphylactic shock may vary. We found, however, that when *Monilia pinoyi* is used as a sensitizing agent, the failure to produce anaphylactic shock through subsequent injection of the specific carbohydrate was quite exceptional, and, therefore, the absence of the reaction in animals cannot be attributed to their individual resistance. From the data in this study, it seems evident that when monilia culture is kept in contact with certain concentrations of formaldehyde ranging from 1:5 to 1:100 its sensitizing power is distinctly diminished. Similar effect has been observed with heat-killed cultures of the same monilia suspended in normal saline and then incubated for some time. From this latter fact, it follows that the sensitizing power of the monilia may be partially destroyed by ageing. It seems, therefore, justifiable to conclude that the effect of formaldehyde upon monilia is comparable in certain respects to its effect upon anatoxins.

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Increased Penetrability of X-Rays Through Normal Lung and Other Air-Infiltrated Substances.

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X-rays are absorbed by a substance through which they pass in proportion, first, to a power of the atomic weight and, second, to the mass of the substance penetrated. This principle is fundamentally concerned in clinical roentgenology, for it permits certain tissues to be distinguished from others on the roentgen-screen and film. The normal lung is a case especially in point. Its average atomic weight is less than that of bone and its mass per unit thickness is much less than that of the neighboring soft tissues, owing to its infiltration with air. The organ is, therefore, more radiolucent than its environs. When air leaves the lung completely and the tissues shrink together, as in atelectasis, the mass per unit thickness becomes approximately the same as that of other soft tissues and the organ may not be distinguishable by x-ray examination from them. However, during certain roentgenologic observations by one of us on specimens of atelectatic lungs, the contrast between the density of atelectatic and air-containing lobes seemed greater than it should be, if atomic weight and mass alone were responsible. The matter was put to the following tests:

A dog was operated upon surgically and the main bronchus of the accessory lobe was ligated. The animal was sacrificed 24 hours later. The lobe was removed and found to be totally airless and collapsed. It was packed into one end of a cardboard cylinder, 3.7 cm. in diameter and 12 cm. in length, and the cylinder was placed upon one quadrant of a roentgenographic film-casset with the lobes next to the casset. The other quadrants were covered with a lead sheet. The ensemble was brought beneath, and 65 cm. away from, a linical x-ray tube, so that the rays could pass longitudinally through the clinder onto the film, and an exposure was made. The cylinder was then inverted on an unexposed quadrant of the casset, with lead covering for the other quadrants, under the tube as before, and an exposure equal to the first was made. The lobe was removed, its bronchus was fitted with a cannula, and its parenchyma was fully aerated by blowing into the cannula. First allowed to deflate, the lobe was reinserted in the cylinder with the cannula protruding

from a window in the side; and it was inflated again, this time not quite completely but as fully as the cylinder allowed. The lobe then occupied about 5 times the length of cylinder formerly occupied. The cylinder and contents were placed upon a new quadrant of the casset, with lead covering for the others, under the tube, and were x-rayed by the same dose. The film was developed. The shadows of the 2 poses of the atelectatic lobe (see A and B in the illustration) were approximately equal in density, and taken as a whole they were distinctly denser than the shadow of the lobe when air-containing (C). The experiment was repeated with another dog and very similar results were obtained (D, E, and F).

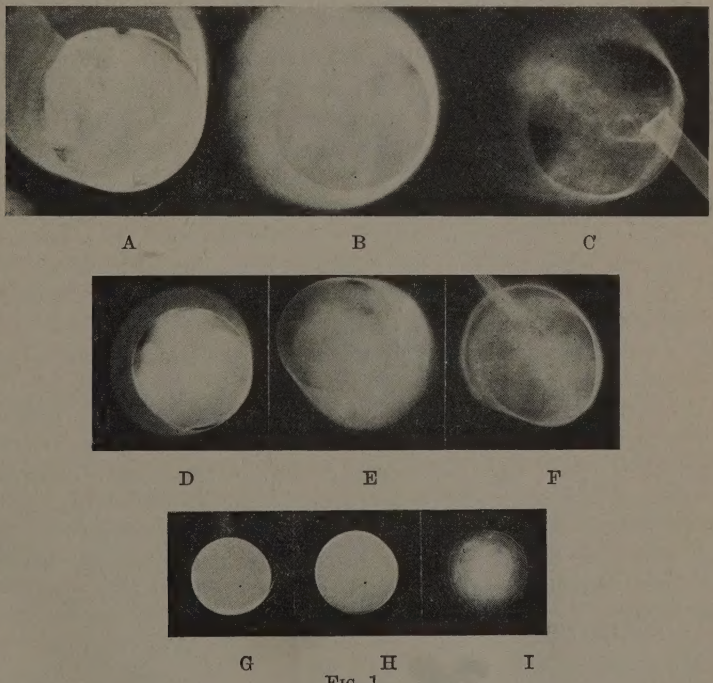

FIG. 1.

Roentgenograms of objects in paper cylinders: upper 2 rows are of lung lobes and lowest row is of glass objects. A, D, and G are of solid objects placed close to the film; B, E, and H are of the same taken a distance from the film; and C, F, and I are of the same close to the film and infiltrated with air.

A disc 0.7 cm. in height and 1.8 cm. in diameter was cut from a bar of glass. The adjacent part of the bar was heated and blown into large bubbles with extremely thin walls. The bubbles were crumpled into a fluffy mass. Two cylinders of the diameter of the

disc and 10 cm. in length were made from heavy paper; and the disc was placed in one end of one and an amount of the fluffy glass equal in weight to the disc was packed into the other. None of the glass sheets were allowed to lie longitudinally. The sheets formed a column 6 cm. high. Now the same roentgenographic procedure was carried out with these objects as described in the cases of the lung lobes. However, the distance of the tube was 175 cm. The result was that the shadows cast by the disc in its 2 poses (G and H) were about equal in density, whereas that from the glass sheets taken as a whole (I) was distinctly lighter. Several other sets of exposures of these subjects gave the same results. The difference in density in each set was not as great as that in each instance with the lungs.

The expansion which followed admission of air between thin septa of the substance, whether lung or glass, occurred in a vertical direction only, so that the same mass was presented to the passage of the rays before and after expansion. The atomic weight could not well have changed. Rearrangement of tissues and increased scattering of rays in the cases of the expanded objects are possible factors in determining the lighter shadows, but we have no adequate explanation of the modes of operation of these factors. The point seems to be demonstrated, however, that the shadows cast by air-infiltrated substances in roentgenograms are lighter than they should be considering only the atomic weights and the masses penetrated by the x-rays.

With the same point in mind, Prof. Wm. S. Halsted is quoted[1] as having called attention to the fact that gas bubbles in the gastro-intestinal tract are often represented in the roentgen-film by contrasts in densities that seem greatly in excess of the displacements of tissue by them. It is also a common observation that parts of chest wall overlying the body of air in cases of pneumothorax appear more transparent than they should considering their thickness.

[1] Holman, E., personal communication to the authors.

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Thrombocytopenic Purpura Hemorrhagica Produced Experimentally with Thorium.

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"Thorotrast"* is a fluid which is said to contain thorium dioxide, 25% by weight, in colloidal suspension and which was developed as an injection medium for clinical pyelo-cystography. Kadrnka[1] also administered the drug intravenously in man and animals and found that the thorium was taken up by the liver and spleen in such quantity as to render these organs radiopaque and plainly visible in the roentgen-film. He formulated a routine method for the purpose which required total dosage of 0.8 to 1 cc. per kg. body weight, given in fractions on several consecutive days. He saw no ill effects from this in most cases—in man occasional vomiting, and once in rabbits persistent hemoglobinuria. The only alterations in the blood were slight transient anemia and leukocytosis, although changes in coagulability were apparently not sought. Except for the presence of granules, believed to be metallic thorium, in the reticulo-endothelial cells of the liver and spleen, no gross or minute lesions were discovered. We are studying further the action of this drug in laboratory animals and report production of a purpuric disease.

Eight rabbits were used. Four were given intravenous injections of "Thorotrast", 0.5 cc. per kg., on 2 consecutive days, and 4 were given larger doses. Three of the latter received a single injection of 3 cc. per kg., and the fourth received 3 cc. per kg. each day for 3 consecutive days. Three of the first 4 and 2 of the second had blood tests immediately before the initial injection and again 4 to 6 hours after the last injection, consisting in determination of bleeding time and clotting time, volumetric measurement of the thrombocytic content of the blood, and observations of clot contractility. Bleeding time was measured according to the method of Duke[2] and the other procedures were those of Van Allen.[3, 4] Symptoms and radiographic

* The method of preparation has not been published. The drug was obtained from Chemische Fabrik von Heyden, Radebeul-Dresden, Germany.

1 Kadrnka, S., *Fortschr. a. d. Geb. d. Roentgenstrahlen*, 1931, **44**, 9.

2 Duke, W., *Arch. Int. Med.*, 1912, **10**, 445.

3 Van Allen, C., *J. Exp. Med.*, 1927, **45**, 69.

4 Van Allen, C., *J. Lab. and Clin. Med.*, 1926, **12**, 282.

effects were also recorded. All but 2 of the rabbits which survived were sacrificed within 24 hours after the last injection. Complete postmortem examinations were made. The histologic and radiographic studies are reserved for another report.

TABLE I.

Data obtained from rabbits before and after treatment with "Thorotrast".

Rabbit No.	Total Dose (cc. per kg.)	Time of Test	Platelets (vol. %)	Bleeding Time (min.)	Clotting Time (min.)	Symptoms	Fate	Gross Findings at Autopsy
1	1	Before	0.85	4.0	7.0	None observed	Living	
		After	0.85	3.0	7.0			
2	1	Before	0.77	3.5	5.0	"	Killed	Hemorrhages in lungs and kidneys.
		After	0.35	3.5	5.0			
3	1	Before	0.82	5.5	7.0	Abortion	"	"
		After	0.3	3.5	6.5			
4	1	No Tests				None observed	Living	
5	3	Before	0.6	2.0	6.5	"	Died	Hemorrhages in heart, liver, kidneys, intestine, and perirenal fat.
		After	0.0	60—	17.0			
6	3	Before	0.95	2.0	6.7	Weakness	Killed	Hemorrhages in lungs, mesentery, and retroperitoneal fat.
		After	0.25	60—	14.0			
7	3	No Tests				None observed	Died	Hemorrhages in brain, lungs, liver, kidneys, and subcutaneous fat.
8	9	"	"	"		Weakness, Hemoptysis	"	Hemorrhages in lungs, heart, subcutaneous tissue. Bloody fluid in pleural, peritoneal, and subarachnoid spaces.

(See accompanying table.) The rabbits with the lowest dose gave no symptoms, except for one, a pregnant female, which aborted after the initial injection. One of the group showed no abnormality of coagulation, but 2 suffered marked reductions in platelets and these had at autopsy petechial hemorrhages in the lungs and renal cortices. None died spontaneously.

Two which received 3 cc. per kg. showed extreme reductions in platelets, marked delays in bleeding time, moderate delays in clotting time, and very slow and incomplete retractions of clot. Weakness

was the only symptom seen, although in the 3 that died the terminal behavior was not witnessed. All rabbits displayed at autopsy extensive extravasations of blood in various internal organs.

The animal with 9 cc. per kg. exhibited marked tendency to bleed from cutaneous abrasions, became weak, and had hemoptysis just before death a few hours after the third injection. Organs and tissues showed extensive hemorrhages.

It is concluded that "Thorotrast", probably due to its content of thorium, given intravenously to rabbits tends to lower the thrombocytic content of the blood and to produce acute purpura hemorrhagica. Amounts 3 or more times the standard dose are usually fatal. The fact that the standard dose was given in 2, rather than in 4 or more fractions as given by Kadrnka, may well account for the difference between our results and his.

5822

Use of Dog Blood Agar for the Differentiation of "S" and "R" Pneumococci.

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In the course of a study on the dissociation of pneumococcus, it became evident that the present method of studying the colony morphology by the use of normal rabbit or horse blood agar plates is not highly satisfactory. This is particularly true in the case of "R" strains derived from Type II pneumococci. When grown on these media the colonies of these "R" strains frequently appear so smooth as to make them almost indistinguishable from their original "S" strains. While many of the "R" pneumococci can be recognized by such a method, yet careful observation and a certain amount of experience and familiarity with the technique, as regarding the degree of light, reflection and magnification are necessary. This report deals with the results obtained by the use of the same medium but slightly modified, the employment of which renders the above qualifications unessential.

It has been found that when making up the blood agar plates (the agar employed has been the ordinary beef infusion agar pH 7.8), if substitution is made of the normal rabbit or horse defibrinated blood, by that of the normal dog, the growth of "S" and "R"

pneumococci on such a medium appears so different in the morphology of their colonies that the differentiation between them becomes extremely simple. With a moderate degree of magnification the "S" colonies are seen to be smooth and shiny, while the "R" colonies, including those derived from Type II "S" pneumococci, reveal a wrinkled and coarsely rough surface (see Plate I). It has also

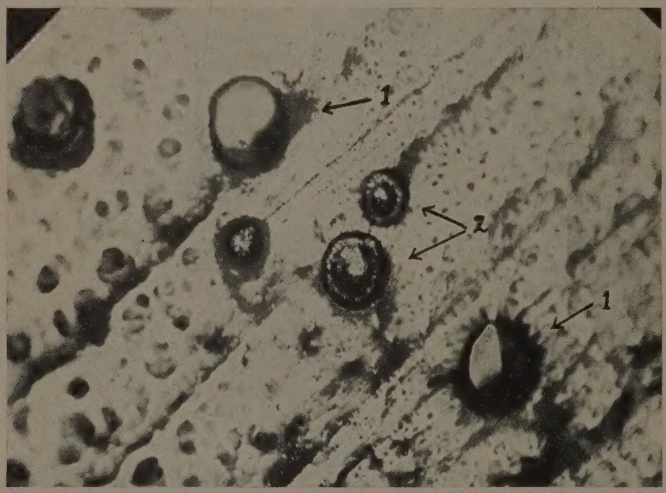

FIG. 1.

1, "Smooth" Pneumococcus colonies. 2, "Rough" Pneumococcus colonies. Mixture of Pneumococcus Type II ("S") and its variant ("R") grown on dog blood agar. × 28.

been observed that pneumococci grown on this medium show marked hemolytic properties. Each colony is surrounded by a wide zone of hemolysis as is seen with hemolytic streptococci.

Defibrinated blood from other normal laboratory animals, such as the guinea pig, white rat, chicken and cat, has also been tried in place of the dog's blood, but none has been found to exert a similar effect on the growth of the "R" pneumococci as that of the dog's blood.

That this property of the dog's blood resides in the cellular elements rather than the plasma is seen in an experiment where dog plasma and washed dog blood cells were mixed with equal parts of horse washed blood cells and horse plasma respectively, and the resulting mixtures employed in making plates. Table I gives the results of growth of "S" and "R" pneumococci on these plates. It is seen that the dog cells are the essential elements which impart to

TABLE I.

Agar plates made with		Differential quality of media for growth of "S" and "R" pneumococci.
Type of plasma	Type of washed blood cells	
Dog	Horse	0
Horse	Dog	+
Dog	———	0
———	Dog	+
Controls		
Dog	Dog	+
Horse	Horse	0

+ Satisfactory. 0 Not satisfactory.

the medium this highly differential character. It is to be noted that even when washed dog blood cells were alone employed in making the blood agar medium, the desired results were equally satisfactory. Similar experiments conducted with rabbit plasma and cells instead of those obtained from the horse, yielded identical results. It has also been observed that the dog cells become hemolyzed rapidly by keeping these dog blood agar plates in the refrigerator for a day or 2, and yet the medium retains its differential qualities. This would point to the fact that intact cells of the dog are not essential, and would seem to indicate that the peculiar quality of the medium depends upon the presence of the hemoglobin of the dog.

The optimum amount of dog's blood in the medium was then investigated. Plates made with varying amounts of defibrinated blood of the dog, constituting 5, 10, 15, and 20% of the blood in the agar, were streaked with "S" and "R" pneumococci and incubated for 16 to 18 hours. It was found that 5% of dog's blood in the medium gives the best result, although the amount of blood as high as 10% is almost as satisfactory; and that when the amount of dog blood is in excess of these quantities, while the "R" pneumococci grow out typically rough, the "S" strains show a tendency to early autolysis as evidenced by wrinkling and flattening of the surface of the colonies. This is particularly true with the "S" forms of Pneumococcus Type III.

The length of incubation of the inoculated plates is also of importance. Incubation at 37°C. for 16 to 18 hours appears to be the optimum for bringing out the differential character of such a medium.

Working with a medium such as described, mixtures of "S" and "R" pneumococci either immediately mixed or grown together before plating, could be readily differentiated and pure cultures of either "S" or "R" forms again obtained. Dissociation of pneumococci ("S"→"R" change) by growth in homologous immune se-

rum has been observed to occur as early as the second transfer. The "R" colonies stand out in marked contrast to the "S" forms and can be easily detected. Tests carried out with the "S" cultures grown on the dog blood agar medium have shown that they fully retain their original biological characteristics such as in regard to specific agglutination* and high virulence, while "R" colonies invariably give the properties of those of "Rough" cultures.

Summary. A highly satisfactory and differential medium for the study of "S" and "R" forms of pneumococci is described. The medium consists of ordinary beef infusion agar pH 7.8 to which 5 to 10% defibrinated blood of the dog is added. To obtain the optimum result, the organisms are plated on such a medium and incubated for 16 to 18 hours. On examination with reflected light the "R" colonies present an extremely rough surface in distinct contrast to the smoothness of "S" forms.

Evidences point to the dog blood cells as furnishing the important constituent of such a medium.

5823

Permeability of Muscle to Sodium Ion.

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It is well known that muscles, like blood corpuscles, contain more potassium than sodium, whereas blood plasma contains more sodium than potassium. The unequal distribution of the cations between the corpuscles and the plasma has been shown experimentally[1] and theoretically[2] to be due to the impermeability of the corpuscle membrane to cations. Although a similar explanation may be offered in the case of muscle, no conclusive proof is available. Mauriac, Aubel and Boutiron[3] found that muscle immersed in experimental edema fluid gained Na but lost K, indicating that the muscle membrane is permeable both to Na and K. The per-

* We are indebted to Dr. A. B. Wadsworth of the New York State Department of Health for the antipneumococcus horse serum employed in this investigation.

1 Doisy, E. A., and Eaton, E. P., *J. Biol. Chem.*, 1921, **47**, 377.

2 Wu, H., *J. Biol. Chem.*, 1926, **70**, 203.

3 Mauriac, P., Aubel, E., et Boutiron, *Compt. rend. Soc. Biol.*, 1927, **97**, 78.

meability to K was confirmed by Wojkczak[4] who found that muscle in nutrient solution containing no glucose lost K. Mond and Amson[5] also found that resting uninjured muscle was permeable to K but impermeable to Na. Callison[6] in a study on the so-called "bound potassium" of muscle found that varying amounts of potassium went into the Ringer solution in which cut pieces of muscle were placed. Thus, while there is general agreement as to the permeability of the muscle to K, the evidence is conflicting with regard to Na. Monauni[7] had reason to believe that even K does not really penetrate through the cells but only through the interstices.

A general criticism which may be raised against all the previous work on the subject is the fact that they are *in vitro* experiments. In the course of a study on the swelling of muscle we had occasion to test the permeability of muscle *in vivo* to sodium, and we have obtained results which, we believe, are conclusive.

Rabbits were anesthetized with morphine hydrochloride. The abdominal wall was slit open, the ureters were tied, and the abdominal wall closed. 20 cc. of 25% NaCl solution were injected into the pleural cavity. After 30 minutes about 40 cc. of blood were drawn from the heart, and pieces of abdominal muscle freed from fat and connective tissue were taken. Determination of H_2O, total base, and chloride were made on the muscle and blood plasma. Water was determined by drying at 110°C. for 12 hours. Chloride was determined by the method of Wilson and Ball,[8] total base by the method of Stadie and Ross.[9]

As controls 20 cc. of 0.8% NaCl were injected into rabbits instead of the 25% solution. The results of 3 controls and 4 experiments are shown in the accompanying table.

It will be seen that the total base, Cl and H_2O contents of the muscle in the control were all normal. In the rabbits receiving injections of strong NaCl solution, the concentration of total base and chloride increased in the muscle as well as in the plasma. The water content is not appreciably changed, showing that the increase of base in the muscle is real and not due to dehydration. It is noteworthy that in the muscle the total base increased by

[4] Wojtczak, A., *Bull. Intern. Acad. Polonaise*, 1927, B, 1253.
[5] Mond, R., and Amson, K., *Arch. Ges. Physiol.*, 1928, **220**, 69.
[6] Callison, W. E., *J. Biol. Chem.*, 1931, **90**, 665.
[7] Monauni, J., *Arch. Ges. Physiol.*, 1929, **221**, 800.
[8] Wilson, D. W., and Ball, E. G., *J. Biol. Chem.*, 1928, **79**, 221.
[9] Stadie, W. C., and Ross, E. C., *J. Biol. Chem.*, 1925, **65**, 735.

TABLE I.
Water, base and chloride in plasma and muscle of rabbit.
(Figures in milliequivalents per kg.)

Rabbit No.	Concentration of NaCl injected	Plasma			Muscle		
		H_2O	Base	Cl	H_2O	Base	Cl
	%						
1	0.8	93.1	165.2	94.5	74.2	124.1	13.7
2		92.7	166.3	101.7	73.9	131.7	11.3
3		92.4	163.3	109.0	75.3	130.7	15.5
Average		92.7	164.9	101.7	74.5	128.8	13.5
4	25.0	93.4	181.5	132.3	75.7	158.0	18.5
5		94.0	188.1	123.2	74.7	153.5	17.8
6		92.1	183.2	133.8	74.6	156.7	17.5
7		93.1	180.7	118.7	73.7	150.8	17.6
Average		93.2	183.4	127.0	74.7	154.8	17.8

26 mM per kg. while the chloride increased only by 4 mM per kg. In the plasma the increases in base and in Cl are not very different. We may conclude, therefore, that the increase of base in the muscle of these rabbits is due to the migration of base from the plasma into the muscle cells and not merely due to increase of the base content in the intercellular fluid. Since plasma contains practically no K, the base which migrates into the muscle must be Na.

The unequal increases of base and chloride in the muscle and plasma suggest a membrane equilibrium and the problem is being studied further. For the present we wish only to conclude that the membrane of the muscle cells *in vivo* is permeable to sodium ion.

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Nutritional Edema. I. Effect of Level and Quality of Protein Intake on Nitrogen Balance, Plasma Proteins and Edema.

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Nutritional edema is apparently another type of edema in which the mechanism of its production is related to the low level of plasma proteins, similar to that of nephrosis and plasmapheresis. In nutritional edema, however, the lowered plasma proteins are brought

about by a deficiency in protein in diet. Denton and Kohman[1] produced edema in a large proportion of rats fed largely on carrots. This work was confirmed by Frisch, Mendel and Peters,[2] who in addition showed that the plasma proteins were low in their rats. Recently Shelburne and Egloff[3] succeeded in producing edema in a dog fed on a low protein diet. We add the following preliminary report of a study on the nitrogen balance, plasma proteins and extent of edema in 2 cases of nutritional edema under the influence of different dietary regimes.

The 2 patients, aged 11 and 20 respectively, were inmates of an orphanage in which they subsisted for a long time on a diet deficient in protein. They presented evidence of undernutrition with marked edema. There was no evidence of cardiac or renal disease. Case 1 had a total plasma protein value of 3.46% and albumin 1.43%. Case 2 had 4.01% for total proteins and 2.17% for albumin. Throughout the 21 periods of 4 days each their total caloric intake was kept at 1400, salt at 6 gm., and water at 1500 cc., while the level and character of the proteins in the diets were varied. Each diet was given for 2 to 5 periods. Diet 1 contained 23.5 gm. (Case 1) and 27.5 gm. (Case 2) of vegetable proteins, estimated intake of protein for them while in the orphanage. With this diet their nitrogen balance was barely maintained, and their plasma proteins showed a slight tendency to increase, and their edema after an initial decrease did not completely subside. With Diet 2 containing practically no protein (0.25 to 0.30 gm. nitrogen per day), there was marked negative nitrogen balance with distinct lowering of plasma proteins and return of edema. Diet 3 containing 26 gm. of animal protein was then administered, resulting in a marked gain in nitrogen, increase in plasma proteins and disappearance of edema. This was followed by Diet 4 in which there were 50 gm. of vegetable proteins. With this diet containing almost double the amount of protein of the previous diet, the extent of positive nitrogen balance was approximately the same. The level of plasma proteins showed a slight tendency to rise in Case 1, and remained about the same in Case 2. Diets 5 and 6 containing 2 gm. and 10 gm. of vegetable proteins respectively were intended to reproduce edema. The result was a marked nitrogen deficit accompanied by a moderate decrease of plasma proteins and reappearance of edema. The lat-

[1] Denton, M. C., and Kohman, E. A., *J. Biol. Chem.*, 1918, **36**, 249.
[2] Frisch, R. A., Mendel, L. B., and Peters, J. P., *J. Biol. Chem.*, 1929, **84**, 167.
[3] Shelburne, S. A., and Egloff, W. C., *Arch. Int. Med.*, 1931, **48**, 1.

ter, however, was not so pronounced as on the previous occasion.

In conclusion it may be stated that nutritional edema is definitely related to the level of plasma proteins which can be easily influenced by the level and quality of protein intake. It seems that 1 gm. of animal protein per kilo is much superior to the same amount of vegetable protein in building up plasma proteins and reducing edema, and that 2 gm. of vegetable protein seem necessary to secure the same effect as produced by 1 gm. of animal protein.

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Nutritional Edema. II. Effect of Alkali and Acids on Nitrogen Balance, Plasma Proteins and Edema.

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The 2 patients reported in the previous paper were studied from the standpoint of the effects of displacements of their blood acid-base balance on nitrogen balance, plasma proteins and edema. They were given known diets containing 1400 calories and enough vegetable proteins to secure nitrogen balance. The salt and water intake was kept approximately constant. Alkalosis was produced by the administration of sodium bicarbonate 12 to 20 gm. per day for one to two 4-day periods, and acidosis by 10% hydrochloric acid 30 cc. per day for 3 days or ammonium chloride 8 gm. per day for 4 or 5 days. There were altogether 3 experiments with alkali, one experiment with hydrochloric acid and one experiment with ammonium chloride with each individual. The results can be summarized as follows:

With each alkali administration, there was definite increase in edema with increase in body weight reaching a maximum within 3 or 4 days. Thereafter the edema began to subside and weight began to decrease in spite of continued alkali ingestion. With hydrochloric acid edema showed a slight diminution in Case 1 and remained unchanged in Case 2. The ingestion of ammonium chloride, however, resulted in a decrease of edema and weight in both cases.

The nitrogen balance in either case remained undisturbed with alkali or acid administration. The plasma proteins showed a slight lowering during several of the periods of alkali adminis-

tration, but practically no change during the remainder. The variations in plasma proteins during acid periods were slight and inconsistent.

The action of alkali in accentuating edema has been noticed by Falta and Quittner[1] in severe undernourished cases of diabetes mellitus and by Albright and Bauer[2] in a case of chronic nephritis of the nephrotic type. The former authors also attempted to produce edema by large doses of sodium bicarbonate in normal individuals without success. The diuretic effect of acids and acid-producing salts like ammonium chloride is well known and has been used in the treatment of various types of edema.

It is surmised here that the ease with which the water balance is so easily influenced by alkali and acid in cases of nutritional edema, nephrosis and severe diabetes with poor nutrition is probably related to the low level of plasma proteins found in such conditions. However, it can not be said that alkali or acid acts directly by altering the level of plasma proteins, as the changes in the latter accompanying alkalosis or acidosis are slight and not consistent.

5826

Small-Flaking or "O" Agglutinin After Intravenous Injections of Typhoid Vaccine.

S. H. ZIA. (Introduced by R. H. P. Sia.)

From the Department of Medicine, Peiping Union Medical College, Peiping.

Since the introduction of the use of prophylactic typhoid vaccine, confusion has risen in interpreting the Widal agglutination reaction in the diagnosis of typhoid fever. Recently Felix[1] introduced the technic of demonstrating the presence of small-flaking or "O" agglutinin, which he claimed to occur only in infection but not after subcutaneous inoculation of the vaccine. Gardner[2] found that while "O" agglutinin can be produced by subcutaneous inoculation of the vaccine, it very rarely reaches a titer higher than 1:200. We wished to know whether this "O" agglutinin can be produced by re-

[1] Falta, W., and Quittner, M., *Wien. klin. Wchnschr.*, 1917, **30**, 1189.

[2] Albright, F., and Bauer, W., *J. Clin. Invest.*, 1929, **7**, 465.

[1] Felix, A., *J. Hyg.*, 1929, **28**, 418.

[2] Gardner, A. D., *J. Hyg.*, 1929, **28**, 376.

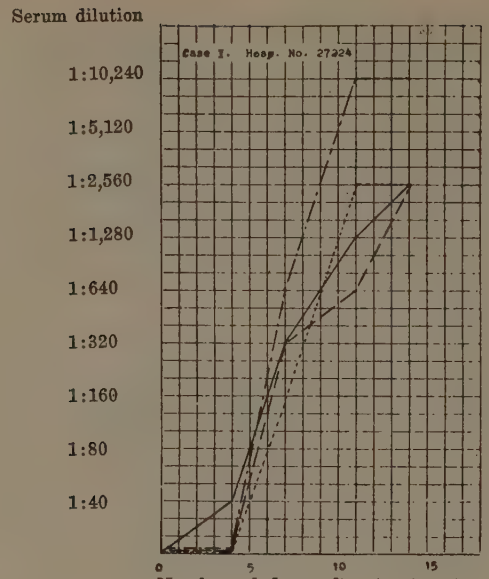
Serum dilution
Case I. Hosp. No. 27224
1:10,240
1:5,120
1:2,560
1:1,280
1:640
1:320
1:160
1:80
1:40
0
5
10
15
Number of days after treatment.

FIG. 1.

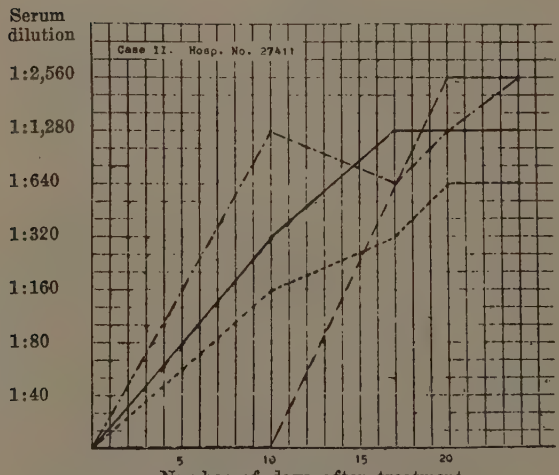
Serum dilution
Case II. Hosp. No. 27411
1:2,560
1:1,280
1:640
1:320
1:160
1:80
1:40
5
10
15
20
Number of days after treatment.
B. typhosus "H" agglutination
B. typhosus "O" agglutination
B. paratyphosus A agglutination
B. paratyphosus B agglutination

FIG. 2.

peated intravenous injections of the vaccine and whether its titer would be higher than that occurring after subcutaneous injections. After this work was begun, we noticed that Pijper and Dau had reported the finding of high titer "O" agglutination after oral ingestion of typhoid vaccine.[3]

Large-flaking or "H" antigens were prepared by the usual method, namely, agar washings in saline or broth of *B. typhosus, B. paratyphosus A* and *B. paratyphosus B* preserved with 0.1% formalin, and used with suitable dilution. Small-flaking or "O" antigen was prepared from *B. typhosus* (Rawlin's strain) treated with alcohol; a modified Bien's method[4] was used which will be described in detail later.

Sera were obtained from patients undergoing treatment for various conditions. These patients all received standard triple typhoid vaccine intravenously with varying numbers of organisms and doses. The first dose was usually 25 million organisms and the usual increase was 25 million with each subsequent injection. All patients showed a systemic reaction with fever and chills. In several instances serum was obtained before the commencement of the treatment and after each injection. In others only single specimens were obtained during treatment.

Dreyer's macroscopic agglutination technic was followed in all the tests. The tubes containing the serum dilutions and the bacterial suspensions were incubated in the water-bath at 37°C. for 18 to 24 hours. In a number of instances incubation at 56°C. over-night was also used and the results with these 2 methods were identical. In every instance the "O" agglutination gave a typical granular flocculation.

The findings of 2 illustrative cases are presented in the accompanying charts. While it is too early to make any definite statement, it is significant that in every case after intravenous injection of the typhoid vaccine, "O" agglutinin was produced in a titer approaching fairly closely to that of the "H" agglutinin. It is therefore reasonable to conclude that "O" agglutinin is readily produced by intravenous stimulation.

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[3] Bien, Z., *Centr. f. Bakteriol,* 1 Abt. Orig., 1925, **93**, 196.

[4] Pijper, A., and Dau, H., *Brit. J. Exp. Path.*, 1930, **11**, 112.

5827

Production of Anti-Sheep Hemolysin in a Donkey.

C. E. LIM AND D. HUIE WONG.

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping.

In the complement fixation test for the diagnosis of syphilis or other diseases, it is usual to use an anti-sheep hemolysin for the production of which rabbits have been employed almost exclusively. Numerous methods have been proposed for the immunization of these animals to produce a high titer serum, but we have found in our routine immunization of such animals that they vary considerably in their individual response to the injections of sheep erythrocytes.

For a routine diagnostic laboratory handling a large number of Wassermann tests where a constant and reliable supply of hemolysin is required, it seems desirable to immunize a larger animal which will yield a supply of hemolysin sufficient to last for a number of years. This will obviate the necessity of immunizing rabbits for this purpose at short intervals and will result in an economy in time and money. Attempts have been made to produce anti-sheep hemolysin on horses but Gilbert[1] was unable to produce it in a horse showing no natural hemolysin in the blood, but was successful with a mule having a small amount of hemolysin before immunization. As these animals are rather costly, it appears to us worth while to find a less expensive experimental animal.

Our choice has accordingly fallen on the donkey, a common beast of burden, which is relatively inexpensive, costing about 20 times the price of a rabbit here, and is of a convenient size for our purpose. Before immunization the blood of the donkey was first tested for the presence of natural anti-sheep hemolysin, but not a trace of it was present. Immunization was then begun by a series of inoculations given through the ear vein. All series of injections were given on 3 successive days at 4-day interval between each series and trial bleedings were taken each day before injection. The first 2 series of injections made with small amounts of washed sheep blood cells in 10 cc. doses of a 10% suspension produced no detectable hemolysin. The third series of injections, consisting of 20 cc. of a 10% suspension of sheep cells followed by 2 injections of the same amount of a

[1] Gilbert, R., *New York State J. Med.*, 1922, **22**, 286.

20% suspension, caused the formation of a small amount of hemolysin (titer 1:120). In the fourth series, the injection of 10 cc. of packed sheep cells caused the animal to have a severe shock, showing signs of weakness of the legs and heavy deep respiration. The titer of hemolysin on a trial bleeding taken on the next day immediately before the second injection of a similar dose increased to 1:800. After the second injection the donkey dropped on the floor and its respiration became very slow and deep. It took one-half an hour for the donkey to recover from the shock. It was, therefore, decided to allow the animal to rest for several days before re-injection. One week later, blood taken before the fifth series of injections was started showed the titer to have risen to 1:2400. The animal suffered no shock from the 3 injections of this series although the dose was increased to 15 cc., but the hemolysin titer rose to 1:4000. Since a sixth series of injections of the same amount of antigen failed to raise the titer, 2 liters of blood were bled from the jugular vein. An immediate drop of the titer to 1:2000 followed the bleeding as indicated in the trial bleeding taken the next day. Another bleeding taken 10 days later caused the titer to drop to 1:1000. The animal became thin and weak and was allowed to rest. Trial bleedings taken once a week showed that the hemolysin titer rose again to 1:2000 without any further injection and remained at this level for 4½ weeks, when another series of injections was started. Ten cc. followed by 2 injections of 15 cc. of packed sheep cells on 3 consecutive days with 4 days of rest between each series were given as before. The titer gradually rose again, reaching 1:3200 within 3 weeks. After the fourth series of such injections in this attempt at restimulation of the antibody production, the titer fell to 1:2000. This would tend to militate against further injections and 2 liters of blood were accordingly bled from the jugular vein. The titer on a trial bleeding taken the next day fell to 1:1000. It rose again to 1:1600 and remained so for 6 weeks, after which the animal was discarded.

One of the main objects in the production of hemolysin is to obtain a high titer serum and to preserve it in such a way as to retain its potency. The highest titer obtained in our experiment is 1:4000 and the average is 1:2000, which compares favorably with that found in the mule by Gilbert. Our hemolysin produced in the donkey was divided into 3 parts for preservation, one part being put up in ampoules aseptically without any preservative, one part put up with 50% neutral glycerol and the third part preserved with 0.02% mercuric chloride. The glycerol and mercuric chloride caused a slight

drop in the titer of the serum immediately after addition, but up to this time (5 months of preservation) none of the serum has showed any additional change. Specimens from all 3 methods have been used successfully in the routine Wassermann tests done in our college hospital for the last 5 months. The supply of hemolysin thus obtained is enough to last us for a number of years and the rates of deterioration in the various methods of preservation will form the subject for further study.

The results reported here suggest the conclusion that anti-sheep hemolysin of a satisfactory titer for use in the complement fixation reaction can be produced in a donkey having no natural hemolysin.

5828

Technique for the Complete Preservation of Supravital Stain of Neutral Red in Paraffin Sections.

C. H. HU. (Introduced by S. H. Liu.)

The technique for the preservation of the supravital stain of neutral red in paraffin sections has been described by McJunkin[1] and Forkner.[2] Their methods enable one to observe most of the neutral red present in the cells; but a certain amount of the stain is inevitably lost in the process of preparation, and they require rapid dehydration and strict limitation of the size of the tissue blocks. The method described below has the advantage over those previously described, in that the entire neutral red stain within the cells is faithfully preserved, that the size of the blocks can be reasonably large, and that the different steps of staining can be carried out in a more leisurely and comfortable manner.

In this technique, advantage is taken of the fact that neutral red is only slightly soluble in aqueous or alcoholic solution containing mercuric bichloride. After the minimal amount of the bichloride required in each solution for the maximal insolubility of the neutral red is determined, any solution which will check further dissolution of neutral red from the tissue can be easily prepared from it.

The tissue to be studied is best stained by the direct injection method described by Forkner with only a slight modification through replacement of his Zenker-formalin by solution A (see below).

[1] McJunkin, F. A., *Am. J. Path.*, 1925, **1**, 305.

[2] Forkner, C. E., *J. Exp. Med.*, 1930, **52**, 379.

For carrying out this technique we use the following 4 different special solutions:

Solution A. Fixing solution, prepared as follows:

Dissolve a small quantity of neutral red in 15 cc. of 40% formalin in a large flat dish. Add to it 85 cc. of Zenker's fluid. Most of the neutral red previously dissolved in formalin is now precipitated out. After a few minutes, during which time the mixture may be stirred with a glass rod, the mixture is filtered for use.

Solution B. Dehydrating solution, prepared as follows:

To every 10 cc. of absolute alcohol add 1.2 cc. of absolute alcohol saturated with $HgCl_2$. Add to this mixture enough neutral red powder so that some of it remains undissolved at the bottom of the bottle.

Solution C. Aqueous solution, prepared as follows:

To 100 cc. of distilled water add 2 cc. of saturated aqueous solution of $HgCl_2$ and then saturate it with neutral red. Filter and use the filtrate.

Solution D. Counter staining solution, prepared as follows:

Saturate the filtrate of solution C with methylene blue powder, filter and use the blue filtrate. This must be prepared fresh each time.

Method. (1) Cut blocks about 1x1 cm. in size and not more than 3-4 mm. in thickness. (2) Fix for 24 hours in solution A. (3) Dehydrate in 3 changes of solution B, one or more hours each time, according to the size of the block. (4) Transfer to xylol, 2 changes, 3-4 hours in each. (5) Imbed in paraffin, cut and attach sections on slides as usual. (6) Remove paraffin from the section with xylol, mount with balsam, or, if counter stain is desired, continue as follows: (7) Wash off xylol with filtrate of solution B. (8) Quickly wash off alcohol with solution C. (9) Counter stain the section with solution D for about 30 seconds. (10) Wash off the excess of methylene blue with solution C. (11) Dehydrate with solution B. (12) Xylol, mount.

From step (7) to step (11) the solutions can be most conveniently used from the drop-bottles.

Southern Section.

Tulane University School of Medicine, November 13, 1931.

5829

Importance of the Spleen as a Reservoir for Red Blood Cells.

E. LAUDA AND E. HAAM. (Introduced by R. Ashman.)

From the Second Medical Clinic of the University of Vienna and the Department of Pathology of the Medical Center of the Louisiana State University, New Orleans.

The fact that different authors vary in their opinion concerning the rôle of the spleen as a reservoir for red blood cells (Radosaljevic and Sekulic, Feldberg and Lewin, and others[1]) has induced us to study this problem.

In the first group of experiments, we studied the effect of intravenous injection of adrenalin on the peripheral blood picture of normal anesthetized dogs. Chloralose was the narcotic chosen because of its dilating effect on the spleen. In 16 dogs the curve of hematocrit determinations (femoral vein) showed an average maximal increase of 13.4%. In a second series of experiments we compared the curves of the hematocrit values of the splenic vein, portal vein and vena cava (or vena femoralis) of dogs after the injection of adrenalin. It was found that a marked but brief increase of the hematocrit numbers takes place in the splenic vein incident to the rapid contraction of the organ. On the other hand, the co-incidental hematocrit values of the portal vein or of the vena cava increase gradually. The curves in Fig. 1 demonstrate clearly the fallacy of single determinations.

In a third group of dogs the tests were repeated under similar conditions but following splenectomy. No or only a slight increase in the hematocrit numbers was found after the injection of adrenalin. In a fourth series of experiments the liver of dogs was completely excluded from the portal circulation by means of a reversed

[1]Radosaljevic and Sekulic, *Wien Arch. f. inn. Med.*, 1930, **20**, 81. Feldberg and Lewin, *Arch. f. d. ges. Physiol.*, 1928, **219**, 246.

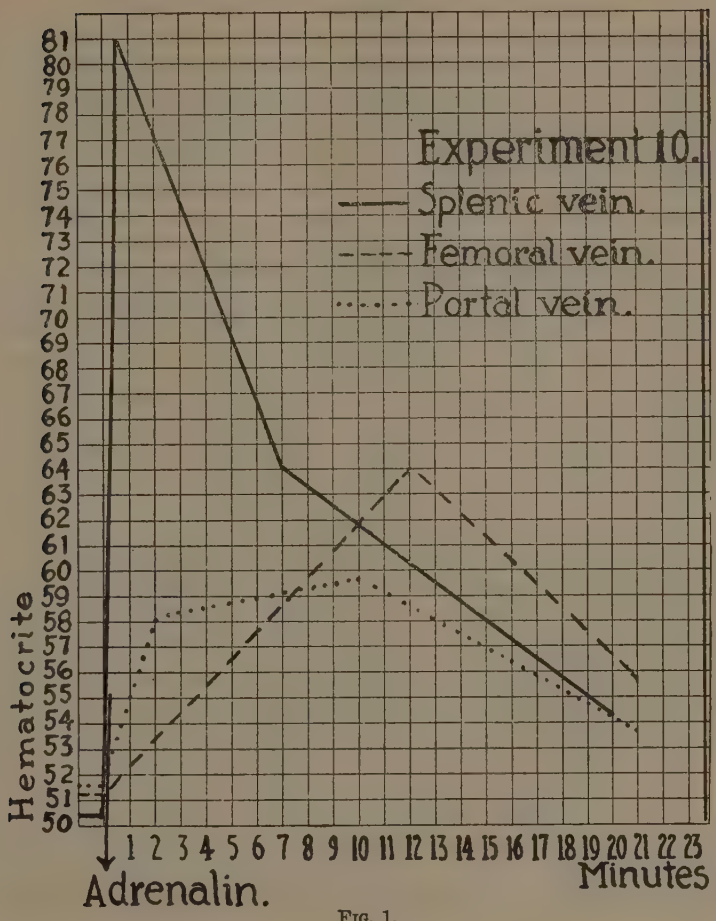

FIG. 1.

Eck fistula. No differences were noted in the curves of the hematocrit values as compared with normal dogs. In a fifth group of dogs fully dilated spleens were extirpated with minimum loss of blood and their blood content determined. It was found that the dog spleen, because of its ability to express stored red blood cells could furnish about 80% of the total increase in the adrenalin erythrocytosis.

Analogous investigations on other animals were made. Twenty rats were killed by means of ether (spleens retain approximately normal size), 10 were given lethal doses of chloralose (producing dilatation of the spleen) and 10 animals were forced to exhaust

themselves completely through swimming (resulting in contracted spleens). The total hemoglobin of the animals of the relaxed and contracted spleens were determined after having placed the extirpated organs into a measured volume of dilute adrenalin solution. Thus a quantitative designation of the function of the spleen as a reservoir for red blood cells was permitted. Our results are listed in Table I.

TABLE I.

Animal group	No. of animals	Hemoglobin in spleen in % of total hgb.	Hgb. of blood expelled from the extirpated spleen by adrenalin:	
			in % of total hgb.	in % of spleen hgb.
A. Chloralose poisoning	10	8.1	3.3	40.3
B. Ether narcosis	20	3.4	0.56	16.6
C. Exhaustion	10	1.7	0.2	11.5

Conclusions. 1. The spleen, though not alone in this function, is the most important blood reservoir in the dog. 2. Chloralose effects storage of red blood cells also in the spleen of the albino rat, while exhaustion leads to ejection of erythrocytes from this organ. The ability of the rat spleen to form a depot of red blood cells is very slight. 3. No generalizations concerning the function of the spleen as a reservoir of red blood cells can be permitted because of the great variability of its function in different animals.

5830

Influence of the Adrenal Glands on the Contractility of the Spleen.

E. HAAM AND H. S. THATCHER. (Introduced by R. Ashman.)

From the Department of Pathology, School of Medicine, University of Arkansas, Little Rock.

The purpose of the experiments was to determine the regulatory mechanism responsible for splenic contraction and dilatation. Tournade and Chabrol[1] believe in a combined nervous and hormonal control of the splenic contractions and Izquierdo and Cannon[2] observed no erythrocytosis after exposure to low oxygen tension in an

[1] Tournade and Chabrol, *Compt. rend. Soc. d. Biol.*, 1924, **90**, 835.

[2] Izquierdo and Cannon, *Am. J. Physiol.*, 1928, **86**, 545.

animal in which the medullary substance of the adrenal glands had been destroyed.

In 20 normal dogs under chloralose narcosis, the hematocrit curve of the peripheral blood was studied after the injection of adrenalin, after short asphyxia and after bleeding. The dogs were then adrenalectomized (2 stage operation) and on 11 of the animals which survived adrenalectomy for more than 2 days, the same tests were repeated and the results compared. It was found that the hematocrit curves after adrenalin injection show no or only a slight decrease in their maximal elevation after adrenalectomy. In contrast to this, the hematocrit values after brief asphyxia and after bleeding did not show the elevation found in normal dogs. In Fig. 1 the curves of hematocrit determinations of an adrenalectomized dog after adrenalin injection and after short asphyxia are shown. Fig. 2 records hematocrit curves obtained after bleeding of adrenalectomized and normal dogs.

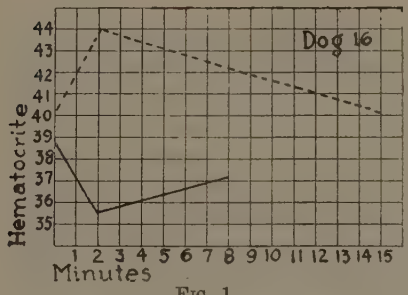

FIG. 1.

The solid line represents the hematocrit curve after brief asphyxia; the dotted line the hematocrit curve after adrenalin injection.

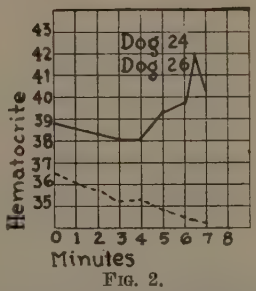

FIG. 2.

The solid line represents normal dog 26; the dotted line adrenalectomized dog 24.

The results seem to point to the assumption that adrenalin is the significant factor responsible for splenic contraction and the resulting erythrocytosis. As it is possible that the polycythemia and the change in the size of the red blood cells after adrenalectomy may be factors which influence the hematocrit curves, one of us is repeating the experiments and studying the contraction of the spleen of adrenalectomized animals *in vivo* by skiagraphic visualization methods.

5831

Effect of Insulin and Glucose on Normal and Obstructed Intestine.*

I. M. GAGE, ALTON OCHSNER AND R. A. CUTTING.

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Since the original experiments of Bulatoa and Carlson[1] concerning the effect of glucose and insulin on the motility of the stomach, a number of similar investigations have been made. Most of the observations have been made on the stomach alone. Quigley and Solomon,[2] however, determined the effect of insulin and glucose on the duodenum of humans and on the colon of dogs. The results of all investigators agree that the administration of insulin causes an increase in gastric and colonic tonus, whereas the glucose causes a cessation of these movements. Because intravenous infusions of glucose are so popular in the treatment of many conditions including intestinal obstruction, the present investigation was undertaken to determine what effect glucose and insulin exert on normal and obstructed small intestine. Eighty-four observations were made on 30 dogs, 20 on the normal animal, 11 on animals with 24-hour obstruction, 22 on animals with 48-hour obstruction, 22 on animals with 72-hour obstruction, and 9 on animals with 96-hour obstruction. Repeated observations on the same animal were made possible by employing the following technic. Under aseptic precautions the terminal ileum was divided and each end inverted by a purse string suture. About 5 cc. proximal to the stump of the proximal end a fenestrated tube was introduced through an enterostomy opening and allowed to extend proximally in the lumen of the bowel for about 20 cm. About 5 cm. proximal to this a second tube carrying a balloon was introduced through another enterostomy opening, the end carrying the balloon being directed proximally, but so placed that the fenestrated enterostomy tube extended beyond the balloon. Both tubes were brought out through the omentum, and the abdominal wall was closed in layers. The animal was allowed to recover completely and after 24 hours the tube carrying the balloon was connected with a Marey tambour and kymographic tracing obtained. Concomitant respiratory tracings were made. Observations were made on the normal unanesthetized animal, following which the enterostomy tube was clamped producing a mechanical obstruction.

* The insulin used in this investigation was kindly furnished by Eli Lilly and Company.

[1] Bulatoa, E., and Carlson, A. J., *Am. J. Physiol.*, 1924, **69**, 107.

[2] Quigley, J. P., and Solomon, E. I., *Am. J. Physiol.*, 1930, **91**, 488.

Observations were made daily as long as the animal survived.

The effects on the motility of the intestine of insulin alone, the combination of insulin and glucose, glucose alone, glucose preceded by insulin, and insulin preceded by glucose were observed. Blood sugar determinations were also made. The results obtained were consistent as regards the action of glucose and insulin on the gut. There was little difference between the reactions of the normal and obstructed gut to the insulin and glucose except that possibly insulin alone exerted a slightly more marked effect on normal gut and that which had been obstructed for 24 hours than that which had been obstructed for longer periods of time. The total results obtained are given in Table I.

TABLE I.

Solution	No. of observations	Increase in intestinal activity	No change	Decrease
		%	%	%
Insulin alone	17	58.8	35.2	5.9
Insulin + glucose	10	40.0	50.0	10.0
Glucose alone	18	0.0	11.1	88.8
Glucose preceded by insulin	37	75.6	13.5	10.8
Insulin preceded by glucose	7	71.4	28.5	0.0

Conclusion. From these results, it is suggested that glucose alone should not be used postoperatively and certainly not in the presence of intestinal obstruction, because glucose alone in the majority of instances exerted an inhibiting effect on intestinal activity. Insulin, or the combination of insulin and glucose produced an increase in intestinal activity. The best results were obtained when insulin preceded the administration of glucose by about one-half hour's time. There was no correlation between the intestinal activity and the blood sugar findings.

5832

Comparative Study of the Rabbit Leucocytic Count Following Injection of Various Antigenic Substances.*

WILLIAM H. HARRIS AND HERBERT J. SCHATTENBERG.

From the Department of Pathology, College of Medicine, Tulane University.

The reactions described here bear a relationship to the subject of nonspecific protein therapy although there is no intention at this time

* Aided by the grant from the David Trautman Schwartz Research Fund.

to consider this phase of the subject. In connection with the study of nonspecific protein reactions, many observations have been reported upon the leucocytic response especially in the human being, and particularly following the intravenous injection of *B. typhosus*. A full review is given by Petersen.[1] Ling[2] reported various blood reactions both in the human and in the rabbit, employing several substances for injection, including milk, peptone, horse serum and *B. typhosus*. Most of the injections were administered intravenously. He obtained only a very slight response in his leucocytic reactions.

We wish to report the comparative leucocytic reactions produced in the rabbit by the subcutaneous, intraperitoneal and intravenous routes of injection for 8 different substances.

Twenty-four full grown rabbits, chiefly males, were used and the following antigens employed: Cysteine, peptone, globulin, market milk, *Staphylococcus aureus, Streptococcus hemolyticus, B. typhosus* and the supernatant portion of a sero-purulent exudate obtained from a streptococcal arthritis. The latter was centrifugalized and the supernatant portion used as such and also after filtration through a Seitz filter. The unfiltered portion was found to contain a few scattered chains of living streptococci.

The bacterial suspensions employed contained approximately one billion bacteria per cc. and were killed with heat at 56°C. for one hour. Heat was used in order to avoid a confusion of the reaction by the addition of a chemical germicide. The other materials were used in a 2% strength with the exception of the milk and arthritic exudate which were employed undiluted. The animals of one series received 2 cc. subcutaneously, in a second series 2 cc. were given intraperitoneally and in the third series 0.5 cc. was administered intravenously excepting for *B. typhosus*, wherein 0.1 cc. was given. Larger amounts of *B. typhosus* given intravenously produced death.

Before inoculation the leucocytic count was made on all rabbits at varying intervals and of 200 estimations made from the marginal ear vein, an average of 8,000 cells per c.mm. was found. This amount is similar to the observations of others as cited in the following article. Our average differential count of the leucocytes was: Neutrophiles, 30.8%; lymphocytes, 64.4%; eosinophiles, 2.2%; basophiles, 2.4%; large mononuclears, 0.2%. We believe that in the estimation especially of total values the phenomenon of local

[1] Petersen, W. F., in Jordan and Falk, The Newer Knowledge of Bacteriology and Immunology. University of Chicago Press, March, 1929, 1086.

[2] Ling, C. Y., *Arch. Int. Med.*, 1925, **35**, 598, 740, 752.

leucocytosis described in the following article must be considered for blood obtained from the ear veins of the rabbit. We noted in some instances that when the ear was pricked repeatedly and the blood obtained with difficulty because of a poor vein, blood collected 6 hours later showed apparently a marked constitutional rise but when checked on the opposite ear, a normal result was obtained.

The results of the inoculations given subcutaneously, intravenously and intraperitoneally are recorded in Table I. From this can be seen the effects produced upon the total and neutrophilic leucocytic count provoked by the antigenic substances.

The counts were made before the injections and subsequent estimations after 6 and 24 hours. In nearly all instances the rise subsided within 24 hours. When it persisted upon the following day,

TABLE I.

Inoculum	Route	Total Count		Neutrophiles × 00	
		Before	After	Before	After
				%	%
Cysteine	Subcutaneously	60	112		*
Peptone	"	54	88		*
Globulin	"	66	72		*
Milk	"	90	86		*
Arthritic Exudate Filtered	"	66	88		*
Arthritic Exudate Unfiltered	"	84	112	30	44
Staph. aureus	"	98	116		*
Streptococcus hem.	"	84	74		*
B. typhosus	"	86	70		*
Cysteine	Intraperitoneally	80	76		*
Peptone	"	78	128	14	21
Globulin	"	58	186	26	42
Milk	"	90	176	24	20
Arthritic Exudate Filtered	"	84	98	28	32
Arthritic Exudate Unfiltered	"	76	176	36	48
Staph. aureus	"	54	336	18	82
Streptococcus hem.	"	64	126	10	44
B. typhosus	"	106	304	24	70
Cysteine	Intravenously	66	116		*
Peptone	"	70	82	46	68
Globulin	"	62	188	28	52
Milk	"	86	208	40	74
Arthritic Exudate Filtered	"	64	80		*
Arthritic Exudate Unfiltered	"	64	164	21	48
Staph. aureus	"	126	218	16	32
Streptococcus hem.	"	48	66		*
B. typhosus	"	80	240	50	84

* Indicates no increase in neutrophiles.

the counts were continued until normal was reached. The recorded results following the injections show the peak reached by the leucocytes and represent the 6-hour period. The counts showing increase were controlled by estimations on the opposite ear.

In the rabbits injected intraperitoneally, the elevation produced by milk and peptone subsided in 48 hours, that produced by unfiltered arthritic exudate in 72 hours while the leucocytosis produced by *B. typhosus* continued for 4 days.

In the rabbits injected intravenously, the increased count subsided in 24 hours for all animals except for unfiltered arthritic exudate, *Staphylococcus aureus, Streptococcus hemolyticus* and *B. typhosus. B. typhosus* produced by this route a leucocytosis lasting over 5 days whereas for the other 3 agents it subsided in 48 hours.

The rabbits injected intravenously and intraperitoneally with *B. typhosus* appear somewhat sick for 24 to 48 hours. The leucocytic reactions in such animals are high and more lasting than for the other substances.

In the rabbits injected subcutaneously only comparatively slight or no reaction was obtained with the dosage employed.

In regard to the differential counts, as shown in Table I, the neutrophiles are increased 7 to 64% in the animals showing the more marked leucocytosis. As might be expected, the greatest neutrophilic increase occurred for *Staphylococcus aureus,* although *B. typhosus* also provoked considerable increase.

TABLE II.
Increment of Leucocytes × 00.

	Method of Injection		
	Subcutaneously	Intraperitoneally	Intravenously
Cysteine	52	—	50
Peptone	34	50	—
Globulin	—	128	126
Milk	—	86	122
Arthritic Exudate, Filtered	22	—	—
Arthritic Exudate, Unfiltered	28	100	100
Staphylococcus aureus	—	182	92
Streptococcus hemolyticus	—	62	—
B. typhosus	—	198	160

— = Increment less than 2,000.

Table II presents the total increment of the leucocytes wherein the rise was over 2,000 cells per c.mm. and also shows the comparative results obtained by each route of injection, *i. e.,* subcutaneous, intravenous and intraperitoneal.

The rectal temperatures of the majority of the rabbits with marked leucocytosis showed a rise of from 1 to 4°.

The tests described demonstrate that of the substances used for injection, consistent leucocytic increase was obtained by intravenous and intraperitoneal administrations of globulin, milk, unfiltered arthritic exudate, *Staphylococcus aureus* and *B. typhosus.* The intraperitoneal injections yield as a group the highest estimations.

These experiments represent preliminary tests to determine certain reactions relative to the resistance of infection, the study of which is now in progress.

5833

Production of Local Leucocytosis in the Rabbit by Mild Provocative Measures.*

HERBERT J. SCHATTENBERG AND WILLIAM H. HARRIS.

From the Department of Pathology, College of Medicine, Tulane University.

Many reports describing the normal leucocytic findings in the rabbit have been published. (Pearce and Casey,[1] Bushnell and Bangs,[2] Kleineberger and Carl.[3]) The estimations of these workers are tabulated below:

TABLE I.

	Total Leucocyte Count	Neutrophiles	Lymphocytes	Large Mononuclear	Eosinophiles	Basophiles
		%	%	%	%	%
Bushnell and Bangs....	10,657	39.1	53.9	0.43	1.1	3.4
Pearce and Casey	9,562	45.4	31.8	10.5	2.2	9.9
Kleineberger and Carl	8,150	50.5	35.0	10.5	1.0	2.5

The findings of Scott and Simon and of Cunningham, Sabin, Sugiyama and Kindwall are cited by Pearce and Casey.

In our observations upon the normal count of rabbits, the blood from the marginal ear vein of 27 normal full grown rabbits, mostly males, was employed. Two hundred counts were made from these animals, and the following estimations obtained:

* Aided by a grant from the David Trautman Schwartz Research Fund.

1 Pearce, Louise, and Casey, A. E., *J. Exp. Med.*, 1930, **51**, 83.

2 Bushnell and Bangs, *J. Inf. Dis.*, 1926, **39**, 291.

3 Kleineberger and Carl, cited by Kolmer and Boerner, Approved Laboratory Technic, 1931, **65**. D. Appleton & Co., New York and London.

TABLE II.

Total Leucocyte Count	Neutrophiles	Lymphocytes	Large Mononuclear	Eosinophiles	Basophiles
8,000	30.8%	64.4%	0.2%	2.2%	2.4%

While estimating these total leucocytic counts, it was noted after several counts had been made on successive mornings that an afternoon check made on the third day showed in some of the animals a very marked increment in the number of white blood cells. Without any apparently sufficient reason, the count made in the afternoon from the ear which had been used to collect the blood, would rise from 15,000 to 25,000 cells per cu.mm. The blood collected from the opposite ear at the time of these increased counts would show a normal result, *i. e.*, a difference of 7,000 to 17,000 cells, with no change in the differential count.

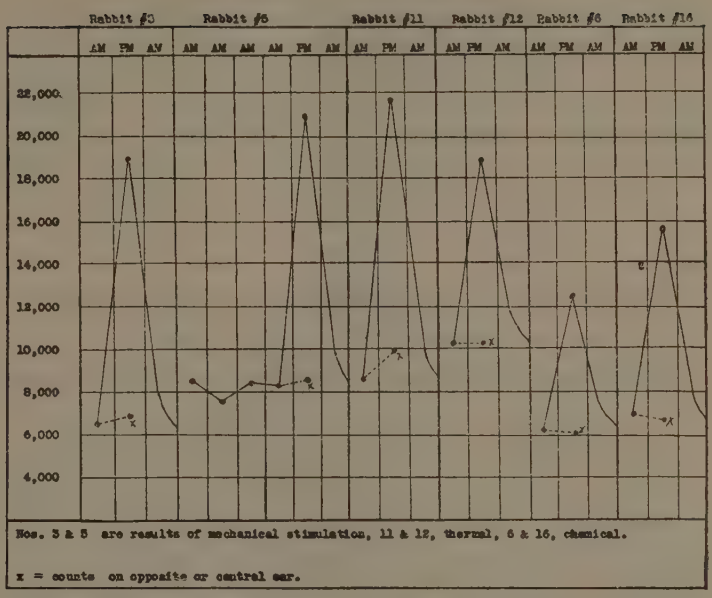
While it is to be expected that any frank inflammatory reaction in a given area will yield a local accumulation of leucocytes with a neutrophilia, it is to be noted that in these animals the ears appear normal and present no cardinal signs of inflammation, and that the blood was collected from the circulatory venous system and not from the more diffuse capillary network.

In the animals wherein the local leucocytosis occurred, several pricks had been necessary to obtain a free flow of blood from poorly shown veins. While no thrombotic blockage was present, sufficient injury to the vascular wall had no doubt occurred and a temporary accumulation of the slowly moving leucocytes resulted. That the condition is temporary is demonstrated by the return to the normal count in 24 hours. The reflex reactions of vaso-dilation and constriction may also play a rôle. Müller[4] believes that the injury to the tissues produces a stimulation of the parasympathetic nervous mechanism. There occurs a vaso-dilatation and a change in the normal condition of the vessel walls, which as a reflex results in an increase of leucocytes in these dilated parts. We have observed that when the veins are well shown and one direct puncture procures a proper amount of blood abnormal rises of the leucocytes do not occur.

To ascertain if this phenomenon could be produced with slight disturbances of the circulatory balance or by mild irritation of the tissues, applications of cold, heat, xylol and alcohol to the ears of 8 rabbits were made, using 2 rabbits for each measure. The warmth

[4] Müller, E. F., *Arch. Int. Med.*, 1925, **35**, 796.

CHART—*Local Leucocytosis.*



was applied by the use of water at 44°C.; cold was applied as ice water; pure xylol and 95% alcohol were employed. These applications were made for 30 minutes each.

The cold and alcohol yielded negative results. Where the warmth was applied an increase of 8,000 and 13,000 cells, respectively, occurred. The xylol application increased the count approximately 5,000 cells. In these animals showing a local leucocytosis, there were increases in the neutrophilic count of from 20% to 30%. All counts returned to normal within 24 hours. The chart reveals a few graphic examples of the extent of the leucocytosis.

In the control rabbits wherein intense heat or prolonged xylol applications were made to the ear producing a marked inflammatory reaction, an increase of 20,000 cells occurred with a neutrophilic increment of between 60% and 70%. In such controls the neutrophilic leucocytosis prevailed during and subsided with the inflammatory lesion present.

The diurnal tide described in the human being and considered by some as due to meal intake, plays no part in these observations, since the control counts on the undisturbed ear reveal normal findings.

It is apparent from these observations that marked variation in

the leucocytic count of the blood obtained directly from the marginal ear vein of the rabbit may be produced, even with causal factors of a mild and apparently harmless nature.

The fact that considerable experimental work on the leucocytic count has been carried out upon the rabbit appears to warrant the report of these observations.

5834

Effect of Activity on the Excitability of Cardiac Muscle.

WALTER E. GARREY AND RICHARD ASHMAN.

From the Department of Physiology, School of Medicine, Vanderbilt University.

In a preliminary study undertaken to ascertain the changes produced in excitability of the excised turtle ventricle by rest and activity, 11 hearts were used from *Chelydra serpentina* and *Emys* sp. The Lapicque potentiometer and chronaximeter were used. The stimuli were applied by way of non-polarizable electrodes.

The results, confirming and extending those previously reported by Garrey and Ashman[1] and by Ashman and Hafkesbring,[2] were variable, but certain conclusions clearly emerge.

1. Excitability, expressed either as rheobase, chronaxie or least time is greatly influenced by activity.

2. In hearts in good physiological condition when excised (3 examples) there was a treppe in excitability, comparable to the treppe in contractility, and the treppe was demonstrable both in rheobase and "least time". Our evidence shows, although less clearly, that there was a treppe in chronaxie. Expressed in terms of quantity of electrical energy required to excite the tissue, activity definitely increased the excitability.

3. In 6 other hearts, also in good or fair physiological condition, it was impossible to demonstrate a definite treppe in rheobase, yet in these the treppe in least time and probably, therefore, in chronaxie was definite or striking. Compared with the resting heart, after several responses the least effective duration of a current of constant strength may be decreased by 20 to 25%.

4. In 2 ventricles the manifestations of fatigue completely masked the treppe. In these the rheobase was markedly raised by

[1] Garrey, W. E., and Ashman, R., *Am. J. Physiol.*, 1931, **98**, 102.

[2] Ashman, R., and Hafkesbring, E., *Am. J. Physiol.*, 1928, **85**, 347.

activity, although the chronaxie was shortened. In terms of the quantity of energy required to excite the tissue, activity here decreased the excitability. This again illustrates a point previously stressed,[3] *i. e.*, that the chronaxie is not necessarily a measure of excitability.

That the observed changes in excitability were not an effect of the local action of the stimulating current was proved by the appearance of similar changes when activity was induced by induction shocks applied to another region of the ventricle.

5835

Specificity of Pathogenic Infections of the Kidney.

H. T. BEACHAM. (Introduced by Alton Ochsner.)

From the Department of Surgery, Tulane University School of Medicine.

From 4 to 6 twenty-four-hour agar slant cultures of colon bacillus and *Staphylococcus aureus* suspended in 3 cc. normal saline were injected transperitoneally into the left renal artery in 21 dogs. According to the length of time the animals survived 6 different groups were recognized as follows: Group 1, two animals survived from 5½ to 6 hours; Group 2, six survived 14 to 18½ hours; Group 3, four survived from 22 to 29 hours; Group 4, two survived 42 to 72 hours; Group 5, four survived 10 to 14 days; Group 6, three survived 16 to 21 days.

A daily record of the temperature and the examination of catheterized specimens of urine was kept. On account of the ease of obtaining sterile urine, multiparous, young animals were used. All animals were autopsied immediately after death to exclude postmortem changes. Sections were preserved in Zenker's solution, and stained with hematoxylin-eosin.

The presence of both organisms in the glomerular capillaries, Bowman's capsule, disproved Crabtree's theory that cocci are secreted by the glomeruli and bacilli by the tubules. All evidence pointed to the fact that blood stream infections produced cortical abscesses while lymphatic infections produced medullary abscesses.

Conclusion. (1) Colon bacilli and staphylococci are commonly present in the urine of so-called normal dogs. (2) Organisms are

[3] Ashman, R., and Garrey, W. E., *Am. J. Physiol.*, 1931, **98**, 109.

very easily demonstrated in the glomerular capillaries, Bowman's capsule, and the tubules within the first 36 hours after injection of the renal artery with 24-hour cultures. (3) Areas in the kidney are found, following injection of the renal artery, in which there is complete necrosis of the tubules, but normal glomeruli and interstitial tissue are present showing that the tubules derive their main blood supply from the efferent vessel of the glomerulus. (4) The injection of one renal artery in this investigation produced no lesions in the contralateral kidney. (5) *Staphylococcus aureus* produced cortical abscesses after 22 hours, whereas the colon bacillus seldom produced abscesses, but resulted in degeneration of the distal tubules when from 4 to 6 agar slants were introduced into the renal artery. (6) Acute glomerulo-nephritis can be produced by injections of *Staphylococcus aureus* and colon bacillus into the renal artery. (7) Chronic interstitial nephritis can be produced within a period of 3 weeks, especially following injection of colon bacilli.

Iowa Section.

State University of Iowa, November 10, 1931.

5836

Arsenic Content of Largemouth Black Bass (Micropterus Salmoides Lacepede) Fingerlings.

A. H. WIEBE, E. G. GROSS AND D. SLAUGHTER.

From the U. S. Fisheries Laboratory, Fairport, Iowa, and the Department of Pharmacology, State University of Iowa.

Domogalla[1] and Surber[2] have used sodium arsenite to control vegetation in lakes and in sloughs respectively. Wiebe[3] has shown that concentrations of arsenic required to kill the vegetation are not harmful to our warm water fish: bass, bluegill, crappie, bullhead, golden shiner, and the common goldfish. The present investigation was undertaken to ascertain whether or not the fish when exposed to arsenic treated water, will acquire and store sufficient arsenic to make them unfit for human consumption.

The investigation shows: (1) That freshwater fish taken from natural waters contain appreciable quantities of arsenic; control fish from the Rock River (Ill.) gave arsenic values ranging from .100 mg. to .425 mg. of As_2O_3 per kg. (2) That fish acquire a certain amount of arsenic from arsenic treated water: the average amount of As_2O_3 per kg. from 9 control fish from the Rock River was .390 mg.; the average for 5 exposed fish was .886 mg. Domestic controls (9 fish) gave a negative test for arsenic. The treated fish (5 individuals) average .703 mg. As_2O_3 per kg.; the range being from .411 mg. to .965 mg. per kg. (3) That the arsenic content in any lot of fish is variable. This fact is brought out by the above figures. (4) There is not exact correlation between the arsenic content of the fish and the concentration of arsenic in the water and the time the fish are exposed; fish exposed to a total of 8 p.p.m. for

[1] Domogalla, B. P., *Eng. News Rec.*, Dec. 9, 1921.

[2] Surber, E., *Proc. Am. Fish. Soc.*, 1921, **59**.

[3] Wiebe, A. H., *Proc. Am. Fish. Soc.*, 1930, **60**.

6 days contained more arsenic than fish that had been exposed to a total of 15 p.p.m. for 42 days. (5) That arsenic is eliminated by the fish. One bass was fed 3 mg. of As_2O_3 on July 22, when analyzed on July 31, 9 days later, only .019 mg. of As_2O_3 were recovered. From another bass that had been fed 2 mg. of As_2O_3, only .0095 mg. were recovered 9 days later. (This may mean also that some of this arsenic, although taken into the stomach, was not absorbed.) Ten fish, all having received the same treatment, were divided into 2 lots of 5 each. One lot was analyzed immediately after the arsenic treatment was discontinued, and gave an average of .799 mg. As_2O_3 per kg. of tissue. The second lot was transferred to untreated water and analyzed after one week. These gave an average value of .586 mg. of As_2O_3 per kg. of tissue.

By comparing our values with those obtained by the Swedish Arsenic Commission, Sodalin[4], from untreated marine fish, we find that our values are much lower. Their values for the cod ranged from 0.5 mg. to 4.1 mg. per kg. (average 1.3 mg.).

5837

Status Marmoratus.

WILLIAM MALAMUD AND K. LOWENBERG.

From the Iowa State Psychopathic Hospital and the State Psychopathic Hospital, Ann Arbor, Michigan.

This syndrome was first established by C. and O. Vogt,[1] who considered as its most characteristic feature the occurrence of quantitatively and qualitatively abnormal myelinated fibers in the striate body, giving it a peculiar marble-like appearance. They considered this condition as restricted to the striate body, and advanced the hypothesis that it was the result of a faulty development. Subsequent findings showed that the original assumptions were not justified. Bielschowsky[2] found similar changes in the cortex, showing that the condition was not restricted to the striate body; Anton[3], Meyer[4], Scholz[5], and others suggested that the lesion might be the

[4] Sodalin, Erick, *Biochem. Z.*, 1928, **201.**

[1] Vogt, C. and O., *J. für Psychol. und Neurol.*, 1920, **25**, 660.

[2] Bielschowsky, M., *J. für Psychol. und Neurol.*, 1924, **31**, 125.

[3] Anton, G., *Jahrbuch. für Psychiat. und Neurol.*, 1896, **14**, 141.

[4] Meyer, A., *Z. f. d. ges. Neurol. und Psychiat.*, 1926, **100**, 529.

[5] Scholz, W., *Z. f. d. ges. Neurol. und Psychiat.*, 1924, **88**, 355.

result of some acquired process (inflammatory or other vascular) rather than a developmental deviation.

Our investigations were particularly concerned with these 2 phases of the problem, and are based on a study of 4 cases. Histological examination of 3 of these left no doubt that the condition was that of *"Status Marmoratus"*, whereas the fourth case, still living, shows a clinical development and picture that makes this diagnosis quite probable. In all of these there was no hereditary predisposition to this disease, and the early development was such that in at least 3 of the 4 there was no reason to suppose that they were not normal to begin with. In all of these the condition seems to have developed following acquired diseases. Histologically too the condition appears to have developed on the basis of acquired processes, most probably inflammatory. In one of the cases, the *"status marmoratus,"* furthermore, was limited to the cortex, none of the lesions being found in the striate body. In this case the condition is chiefly localized around blood vessels which show signs of a previous inflammatory process.

These results would justify the conclusion that (1) the condition is not necessarily restricted to any special system in the brain and (2) it should be looked upon as the result of a process disease and not only a developmental anomaly. This latter would bring up the question of the origin of the myelin bundles. Three possibilities were advanced by Meyer,[4] viz., (1) actual regeneration of myelinated fibers, (2) development of a myelin covering around previously naked axis cylinders, (3) mechanically conditioned arrangement of preexisting myelinated fibers. In our studies the fine and delicate nature of the fibers, their abundance in places where they are not normally found in such quantities, their poorer staining in the Weigert method, and their relationship to the glial fibers within the scars leads us to the conclusion that they are produced by actual regeneration, but, that their peculiar arrangement into bundles is, at least partly, conditioned by mechanical factors of the scar formation.

5838

Sex of Parabiotic Twins of Ambystoma Maculatum (Shaw).*

E. WITSCHI, W. GILBERT AND G. O. ANDREW.

From the Zoological Laboratory, State University of Iowa.

Since Burns published his paper on "the sex of parabiotic twins in amphibia,"[1] it has been found difficult to reconcile his statements and his general conclusions with the results of other experimental studies in sex development of vertebrates. He alleged that everyone of 80 pairs of *Ambystoma maculatum* joined in parabiosis at the tailbud stage had developed into unisexual twins, 36 being of the female and 44 of the male sex. The absence of heterosexual combinations was said to suggest that embryonic sex differentiation is a hormone controlled reaction of the "all or none" type. Moreover, the approximate 1 : 1 ratio of male and female pairs was taken as an indication "that there is no prepotency constantly favoring either sex," but that in genetically heterosexual pairs the first differentiating member governs also the development of its mate so as to develop into the identical sex. In contrast to this, Witschi[2] and Witschi and McCurdy[3] found in parabiotic frogs and newts a clear ratio of 1♂♂:2♂♀:1♀♀. In cases of secondarily induced sex reversal the male sex predominated. Later Burns[4] reported new experiments with *Ambystoma tigrinum* that gave results corresponding closely to ours. However, he still upholds the accuracy of his original statements with respect to *Ambystoma maculatum.* Differences of such fundamental character within one genus seemed extraordinary and since they were an obstacle to a rational interpretation of the whole body of experimental data, the authors were moved to repeat Burns' experiment with the same material—*Ambystoma maculatum* (Shaw), *syn. A. punctatum* (L) of New Haven, Connecticut. Though the investigation which was started in the spring of this year is not yet completed, it is already clear at this time that Burns must have misinterpreted his material. Of 41 pairs so far preserved nearly one-half represent heterosexual combinations. The result is 7♂♂:18♂♀:16♀♀.

* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

[1] Burns, R. K., *J. Exp. Zool.*, 1925, **42**, 31.

[2] Witschi, E., *Biol. Bull.*, 1927, **52**, 137; *J. Exp. Zool.*, 1931, **58**, 113.

[3] Witschi, E., and McCurdy, H. M., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 655; Witschi, E., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 763.

[4] Burns, R. K., *J. Exp. Zool.*, 1930, **55**, 123; 1931, **60**, 339.

In the heterosexual pairs the following conditions are met with: (1) In 3 cases the female had developed faster than the male twin; consequently typical ovaries (though of slightly subnormal size) are present near the end of the larval period. (2) In several cases (about 6) the ovaries were topographically typical, but the cortex contained a reduced number of germ cells of which few or none had entered the ovocyte stage. (3) In nearly half the cases (8) the ovaries were only small vestiges, sterile, or containing few abnormal germ cells (free-martin effect). (4) Feeble attempts at sex reversal were observed in only 2 of these female gonads. (5) the testes of the male twins are at best slightly smaller. In some cases (about 4), however, they were found considerably smaller than in ♂♂ pairs of corresponding size and age, or in single controls. In this respect *Ambystoma maculatum* resembles more closely the conditions previously reported for frogs (Witschi[2]) than those of the California newt (Witschi and McCurdy[3]). In the latter a more decided reciprocal inhibition of the testicular development has been found. In every case, however, facts are in disagreement with Burns'[4] conclusion that "the male usually dominates because of a differential stimulative effect of the anterior hypophysis accelerating development of the testis"—the latter supposedly being "doubly stimulated" in heterosexual pairs. (6) Male twin mates of females with relatively well developed ovaries in a few cases (4) exhibit hermaphroditic features not observed to this extent in the 7 ♂♂ pairs nor among the single controls (58 males plus 41 females). As in some similar cases of heterosexual pairs of frogs (Witschi[2]), the early developing ovaries obviously have retarded the testicular differentiation of the co-twin, giving its cortex a chance to develop in the female sense. No trace of male-female sex inversion, i. e., no transformation of testicular into ovarial tissues has ever been observed.

Minnesota Section.

University of Minnesota, November 18, 1931.

5839

Heparin Inhibition of Coagulating Agents Rendered Isocoagulant.

JOSEPH T. KING. (Introduced by F. H. Scott.)

From the Department of Physiology, University of Minnesota.

In attempting to use serum rather than tissue extract for the coagulant in clotting heparin-plasma it was found that frequently a plasma rendered incoagulable with heparin could not be clotted with serum but could be clotted with tissue extract. This observation suggested that the coagulant in serum (thrombin) might be more sensitive to heparin than the coagulant in tissue extract (tissue fibrinogen).

One must consider the possibility of the concentration of coagulant in the tissue extract being greater than in the serum. This factor was controlled by rendering the 2 coagulants isocoagulant by dilution of the stronger of the 2 with physiological saline. They were regarded as isocoagulant when similar amounts of either shortened the coagulation time of a recalcified citrate plasma to the same extent.

The citrate plasma is from adult rabbits, citrated to 0.5%; serum is obtained from the clotted recalcified citrate plasma.

The tissue fibrinogen was kindly furnished by the Wm. S. Merrell Co. ("Fibrogen" for subcutaneous use).

In Experiment A. Table I, the plasma has a fairly long recalcification time of 13½ minutes; this is reduced to 3 minutes by 4 drops of either coagulant. The heparin concentrations are high at the start being reduced by dilution, the clotting time becoming consistently shorter for both coagulants. In each set the serum is more inhibited than the tissue fibrinogen. Control at the end shows the coagulants to be isocoagulant and within 15 seconds of the original value.

In Experiment B, the plasma has a recalcification time of 3½

TABLE I.

Citrate plasma and calcium chloride 1%, 2 drops in each test.

Exp.	Tissue Fibrinogen	Serum	Heparin	Water	Coag. Time	Remarks
A				6	13½ min.	Recalc. time plasma No. 136
	4			2	3 "	Isocoagulant pair
		4		2	3 "	
	4		1	1	22½ "	
		4	1	1	None 1 hr 42 min.	
	4		1	1	6½ "	Heparin diluted 1:3
		4	1	1	None 2 hr. 31 min.	" " "
	4		1	1	4¾ "	" " 1:5
		4	1	1	36+ "	" " "
	4		1	1	4¼ "	" " 1:9
		4	1	1	21 "	" " "
	4			2	3¼ "	Control on isocoag. pr.
		4		2	3¼ "	
B				6	3½ "	Recalc. time plasma No. 139
	2			4	2 "	Isocoag. pr.
		2		4	2 "	
	2		1	3	2 "	
		2	1	3	3¾ "	
			1	5	5¼ "	
	2		2	2	3¾ "	
		2	2	2	7 "	
			2	4	8 "	
	2		3	1	6¾ "	
		2	3	1	14¼ "	
			3	3	20½ "	
	2		4		10 "	
		2	4		30 "	
			4	2	50+ "	

minutes which is shortened to 2 minutes by either coagulant. The heparin concentration is here being increased; the effect of heparin on the recalcified plasma without any coagulant is also shown for each set. There is a consistent lengthening of the clotting time as heparin is increased. In each set serum is more inhibited. In each the order of clotting is tissue fibrinogen, serum, no coagulant. Serum, therefore, though strongly inhibited still exerts some effect; this is particularly noticeable in higher concentrations of heparin.

Some irregular results have been noted which suggest that tissue fibrinogen undergoes some change on standing after dilution which renders it unsuitable for use and that sera which fail to accelerate clotting after high dilution are also unsuitable.

The pipettes used in these experiments are calibrated to drop the same number of drops per cc. when held at the same angle and drop-

ping at the same rate. The concentration of blood fibrinogen is kept constant by working with a total volume of 10 drops.

These data are tentatively interpreted as indicating that thrombin is more sensitive to heparin inhibition than is tissue fibrinogen. The possibility that tissue fibrinogen suspension used might in some non-specific manner inactivate the heparin has to be considered.

5840

Volume of the Various Lobes of the Hypophysis During Pregnancy in the Rat.

SAM STEIN. (Introduced by A. T. Rasmussen.)

From the Department of Anatomy, University of Minnesota.

Contrary to the general tendency of the hypophysis, and particularly the anterior lobe, to enlarge during pregnancy in many animals, it appears from the data on 86 albino rats tabulated below, that the organ, as a whole, does not enlarge. If anything, the whole organ is slightly smaller in the normal pregnant animals. No significant difference was found in the relative volume of the various lobes as determined by the paper weight method on serial sections.

TABLE I.

Condition of Animals	No. of animals	Average age	Average body length	Average body weight	Average weight whole gland	Relative Volume of Lobes		
						Pars Anterior Average	Pars Posterior Average	Pars Intermedia Average
		days	cm.	gm.	mgm.	%	%	%
Group I—Normal non-pregnant controls	20	109	20.0	185	10.67	87.42	7.73	4.86
Group II—Normal pregnant	28	135	19.9	175*	10.14	86.67	8.45	4.81
Group III—Pregnant vitamin E deficient	19	136	20.3	229	12.41	87.39	7.78	4.82
Group IV—Pregnant cured of vitamin E deficiency	19	153	20.3	241	12.79	87.77	7.60	4.61

* When impregnated.

All the animals used in these investigations were standard female albino rats reared under uniform conditions in the animal colony of the Department of Anatomy, University of Minnesota.

Group I—Normal non-pregnant controls were specifically chosen litter mates of the animals in Group II. They were all killed at approximately the same body weight of 185 gm.

Group II—Normal pregnant group—Litter mates of Group I were impregnated at about 175 gm. At least one animal was killed on each day of the gestation period, which in a rat is 22 days. Impregnation was done at this age on the assumption that they would have attained an average body weight similar to group I when killed if pregnancy had not been induced.

Group III—Consisted of a group of pregnant animals retained on a diet deficient in the anti-sterility vitamin E, after having passed through one gestation period with a diet deficient in vitamin E.

Group IV—Is a group of pregnant animals which was fed vitamin E after having passed through one gestation period with a diet deficient in vitamin E. This procedure did not apparently modify the relative volume of the lobes of the hypophysis as compared with group III.

5841

Avertin as an Anesthetic During Experimental Operations on Central Nervous System of Cat.

G. L. RASMUSSEN. (Introduced by A. T. Rasmussen.)

From the Department of Anatomy, University of Minnesota.

In operations on the central nervous system in cats, we have found that Avertin has many advantages over such an anesthetic as ether. We have used it on about 100 animals and find it to be of special value in several respects.

1. Being given per rectum, the dangers of contamination by the ether mask are eliminated when operating on the head region.

2. The animals may be made to remain perfectly quiet through the entire operation even if lasting up to 5 hours, without the use of any other drug, thus reducing the number of assistants—an anesthetist not being needed. It is therefore especially valuable where long periods of immobility are required, as in experiments where electrodes are to be kept for a considerable time in exactly the same place.

3. The increase of flow of mucus and saliva which is often produced by ether is eliminated by the use of Avertin. This feature is

especially desirable in operations such as those on the base of the skull, in which the trachea must be retracted to make good exposure.

4. The danger of tongue swallowing is eliminated since Avertin induces the tongue to drop forward.

5. After the use of Avertin the animal sleeps quietly for 24-48 hours. This period of rest encourages wound healing and reduces the danger of hemorrhage and infection, since the animal cannot open the wound.

We found it convenient to etherize the animal before administering the Avertin. Because of this fact, it is necessary to take special precaution against over-dosage due to the combined effect of ether and Avertin. The method which we found advantageous is to etherize the animal only to the point where it can be tied down to the operating board without resistance. Then ⅓-½ of the usual dosage of Avertin for cats (which is 300 mg. per kilo of body weight) is given, depending on the depth of narcosis produced by the ether. Later, when the animal begins to awaken, ½ of the remaining Avertin is given. Shortly after this dosage, the animal usually passes into a state of narcosis. The remainder of the dosage is given only if the animal shows signs of awakening during the operation, or it may be given at the end of the operation in order to keep the animal asleep for a long period following the operation.

In a few cases of respiratory failure due to over-dosage, artificial respiration usually restored the animals.

New York Section.

New York Academy of Medicine, December 16, 1931.

5842

Influence of Secretions of the Upper Respiratory Tract on Tissue Resistance.

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Duran-Reynals[1] and others have shown that extracts from certain organs such as testicle greatly enhance the invasion of tissue by various bacteria and viruses. This can be demonstrated by injecting into the shaved skin of a rabbit a living suspension of pathogenic bacteria mixed with the organ extract to be tested and at another site an equal number of bacteria in plain broth or saline. After 24 hours a comparison of the size of the lesions and the relative degree of necrosis furnishes an index of the enhancing rôle of the tissue extract.

We have investigated a similar effect exerted by secretions from the upper respiratory tract. In most of our experiments we have employed a virulent strain of *B. lepisepticum* (R. D.) as this organism is quite pathogenic for rabbits and produces a slowly progressing cutaneous lesion which has become familiar to us from previous experiments. The experimental material was obtained by irrigating with plain broth the nasal passages of various individuals both normal and those suffering from acute colds. This material was passed through Berkefeld or Seitz filters and tested aerobically for sterility. This was then concentrated by distillation *in vacuo* to approximately one-fifth the original value and preserved in the ice-box with cystein 0.25% under vaseline seal. In other cases, fresh material was used without concentration. As control some of the broth was treated in an identical manner. In most of our experiments .2 cc. of the washings were mixed with .1 cc. fresh, thin saline

[1] Duran-Reynals, *J. Exp. Med.*, 1929, **50**, 327.

emulsion of *B. lepisepticum* and .2 cc. of this mixture injected into one flank of a rabbit while in the opposite side an identical preparation of organisms and control broth was injected. In approximately 40 experiments it has been noted that with the presence of nasal excretions the areas of infection were much larger and more necrotic than those where control material was used. Within a few hours the areas receiving the washings were more puffy and tended to gravitate more readily down the flank of the animal, indicating that a local œdema and cellular alteration is probably the cause of the phenomenon. The infection spreads rapidly on the infected side and usually causes the death of the animal in about 5 days.

The nasal secretion will not only facilitate the spread of fresh infection, but when injected into a healing quiescent lesion will frequently light up the process, causing extension of the infection. Its action is also striking in immune animals where ordinarily the bacteria produce only trivial lesions when injected into the skin, but when mixed with nasal secretion, produce large necrotic lesions which, however, do not spread beyond the limits of the initial infection and do not cause death of the animal.

The nasal secretions of various individuals differ in the capacity to enhance infection. Material obtained from some persons apparently free of respiratory infection showed this action. However, washings obtained during an acute cold, in most instances, accelerated infection more than those taken during a normal period. This phenomenon seems to be analogous to that described by Reynals for tissue extracts and is chemical in nature. It probably does not depend upon the filterable agent described by Dochez and his co-workers[2] which when inoculated into human volunteers produces colds. Dr. Dochez has very kindly furnished us with tissue cultures containing this agent and no consistent spread of infection was produced by material presumably active. Saliva showed the effect to a moderate degree but tears were inert. Washings from rabbits with acute snuffles are as active as those obtained from humans. Heating at 80° C for ½ hour decreases but does not totally destroy the action.

Further work is being done to determine whether nasal secretion may play an enhancing rôle for organisms transmitted from one individual to another in the course of natural infection.

[2] Dochez, A. R., Mills, K. C., and Kneeland, Y., Jr., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 513.

5843

Biological Properties of "Fresh" and "Stock" Strains of the Meningococcus.

GEOFFREY RAKE. (Introduced by L. T. Webster.)

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A study has been made of the biological differences in freshly isolated and old stock strains of the meningococcus. The majority of freshly isolated strains present colonies on blood agar which are pearly in color, lenticular and flattened in form, round in outline, and distinctly moist. While single colonies measure up to 4 mm. in diameter after 24 hours' growth, there is a tendency to coalesce with the formation of a sheet-like growth. By the end of 36 hours the original growth tends to become transparent and glassy, sometimes with the formation of minute, glistening crystals on the surface; new and opaque, yellowish white growth appears at the margin usually in the form of papillæ, and this tends to grow over and obliterate the original glassy colony. While, save rarely, transplantation from the original growth is unsuccessful, subculture can be made from the marginal growth during the next 48 or 72 hours. The colonies so obtained are usually more opaque and domed than in the original culture. Individual colonies are mostly smaller and show less tendency to coalesce, but there are some larger, flattened colonies resembling those of the original culture, giving the plate a characteristically uneven growth. Further subculture accentuates those characteristics noted for the secondary growth—the colonies become smaller and more domed with less tendency to coalesce. Most of the older strains show opaque white colonies but in occasional stock strains the colonies are transparent. They remain viable on blood agar for 48 or 72 hours. In many the formation of crystals becomes well-marked at the end of 48 hours and subcultures are no longer successful. Rough forms have appeared in only one strain,—a stock Type IV culture with considerable yellowish pigment formation. These colonies present a definitely wrinkled surface and emulsify with difficulty in saline; they resemble the colonies of *Micrococcus pharyngeus siccus* but tend to revert readily to the smooth form. Freshly isolated strains show no hemolysis after 24 hours' growth, though there may be some at the end of 48 hours. Older strains often show quite marked hemolysis within 24 hours.

Many investigators have suspected the presence of a capsule in

the meningococcus but Baker[1] alone has shown its existence with stained preparations. By using a modified Baker capsule stain, well-marked encapsulation can be demonstrated in freshly isolated strains; in older strains this capsule becomes progressively less and the rough form shows no capsule.

The acid agglutination range of the meningococcus fails to show the clear cut picture found with many other organisms. Fresh strains, however, do differ from older and more especially from the stock strains. Freshly isolated strains show a relatively narrow zone, ranging from about pH 3 to pH 5 with occasional strains going down to pH 2.7 and a very few up to pH 5.6. After several transplants the zone becomes broader and moves to the acid side, most cultures agglutinating between pH 2.1 or pH 2.3 and pH 4.7, while some go out to pH 5. The stock strains show marked irregularity. The zone is still broader and the majority of strains agglutinate from pH 2.1 or pH 2.3 out to pH 5, pH 5.6, or even pH 6.3.

The range of acid agglutination explains the occasional appearance of salt sensitivity amongst fresh cultures and its more frequent occurrence in the stock strains. Owing to the absorption of CO_2 from the air, saline solution prepared from ordinary distilled water gives a pH between 5.5 and 6. This is within the range of agglutination of occasional fresh strains and several stock strains.

Both protein and carbohydrate fractions can be obtained from a solution of lysed meningococci.* The carbohydrate portion can be separated into 2 parts. One, the more abundant of the 2, is precipitated by about 8 volumes of 95% alcohol. It is readily redissolved in distilled water and can be freed from non-carbohydrate substances by repeated precipitation. It is neither type nor species specific, giving a precipitate with antimeningococcal, antigonococcal and antipneumococcal (especially Type III) sera. It does not precipitate with antistreptococcal sera or with sera prepared against the typhoid and dysentery organisms.

The second carbohydrate fraction has been obtained from fresh strains in smaller amounts. It is precipitated from solution by 1½ to 2 volumes of 95% alcohol and is readily soluble in distilled water so that it can be purified by repeated precipitation. It is type specific, giving a precipitate with some samples of antimeningococcal polyvalent sera, while the fractions obtained from each of the 4

[1] Baker, S. L., *Brit. J. Exp. Path.*, 1920, **1**, 127.

* Zozaya has recently reported a non-specific polysaccharide for this organism (*J. Exp. Med.*, 1931, **54**, 725) and Przesmycki (*J. Inf. Dis.*, 1924, **35**, 537) has briefly described the isolation of a type specific carbohydrate.

types show, in high dilutions, precipitation by the homologous type serum. This fraction corresponds therefore to the S or soluble specific substance of other organisms.

5844

Immunological Differences Between a Strain of Monkey Virus and Human Poliomyelitis Virus.*

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Having succeeded in producing potent antiserums to monkey poliomyelitis virus by the injection of horses with increasing doses of a suspension of infected spinal cords, the occasion of the recent epidemic of polio in New York City has made it possible to determine the potency of these antiserums as neutralizing substances for freshly isolated strains of virus. It has been shown[1] that concentrates from these antiserums were of very high titre and were effective in protecting monkeys already infected with poliomyelitis virus of monkeys as well as bringing about virus neutralizations *(in vitro)* in very high dilution. In this respect these serums were approximately 5 times as potent as human convalescent serum. With continued injection of horses and continued passage of the virus through monkeys a serum has been evolved which neutralizes monkey virus in a dilution upward of 1:500 in spite of the fact that the virus is now more infective for monkeys than it was previously (5% virus, M.L.D. 0.05 cc.) while human convalescent serum neutralizes

TABLE I.
Amount of Antiserum Required to Neutralize.

	Human Convalescent Serum	Horse Concentrate
Monkey Virus No. 1 in 1930, 5% emulsion	20:1	100:1
Monkey Virus No. 1 in 1931, 1% emulsion	5:1	200:1
Monkey Virus (Nasal) No. 2 in 1931, 5% emulsion	20:1	500:1
Human Virus 1931 epidemic, 5% emulsion	50:1	20:1

* Aided by a grant from the Leopold Schepp Foundation.

[1] *J. Exp. Med.*, 1931, **53**, 553.

this virus in 1:20 only. On the other hand, human strains recently isolated fail to be neutralized in as low as 1 : 20 by the otherwise powerful serums. Convalescent serum from past epidemics neutralizes the new human strains in dilutions up to the neighborhood of 1 : 50. These relative values may be set down in tabular form. (See Table I.)

5845

Nutritive Properties of the Seed of the Tobacco Plant (Nicotiana tabacum)*

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(With the Cooperation of Helen C. Cronin and Elizabeth C. Callison.)
From the Laboratory of Physiological Chemistry, Yale University, and the Biochemical Laboratory, Connecticut Agricultural Experiment Station, New Haven.

Although every part of the tobacco plant has been reported to contain nicotine, this alkaloid could not be detected by Vickery and Pucher in the fully ripened seed of Connecticut shade-grown tobacco by chemical methods.[1] Ilyin,[2] who has studied the distribution of nicotine in the plant, found that immature seed, and particularly the ovules at an early stage of development, contained small proportions, but that, as ripening progressed, the alkaloid content diminished until finally none could be demonstrated. In view of the toxic properties of nicotine it seemed that a simple physiological test for its presence in tobacco seed would consist in feeding trials on small animals. We therefore offered to albino rats a ration that consisted either of ground tobacco seed 98%, Osborne-Mendel salt mixture[3] 2%, or ground tobacco seed 99%, sodium chloride 0.5%, calcium carbonate 0.5%; cod liver oil was administered as a supplement at the rate of 10 drops per day. The diet was consumed with avidity and without any evident untoward consequences; the animals grew at a satisfactory rate and appeared to be normal in every respect. This somewhat surprising outcome led to a detailed study of the nutritive properties of the tobacco seed.

* The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[1] Vickery, H. B., and Pucher, G. W., *Conn. Agri. Exp. Station*, 1930, Bull. 311, 234.

[2] Ilyin, G., *U. S. S. R. State Inst. for Tobacco Research*, 1929, Bull. 57.

[3] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, **37**, 572.

The seeds are very small, 100 of them weighing in the neighborhood of 0.009 gm., and yield approximately 43% of a pale yellow oil when extracted with anhydrous ether. The crude protein, calculated from the nitrogen content, amounts to approximately 20%. At least half the protein of the seed can be obtained as a crystalline globulin that resembles edestin from hemp seed in many respects.

Experiments designed to provide evidence of the presence of vitamin A in the seed were not entirely conclusive. It is certain that the seed does not contain a concentration of this vitamin adequate for successful growth, or for complete protection against xerophthalmia; it is probable, however, that the vitamin is not entirely absent.

Vitamins B and G were found to be present in tobacco seed in quantities sufficient to promote growth at a normal rate and to provide for general well-being. Under circumstances in which unusual demands for vitamins B and G are made by the organism as, for example, during lactation, a moderate degree of deficiency was apparent.

No final conclusion can yet be drawn with regard to vitamin D, but the available evidence indicates that tobacco seed is almost entirely deficient in this respect.

Vitamin E is present in tobacco seed in quantities adequate to provide for reproduction and to protect both sexes from physiological changes due to a deficiency of this factor. Out of many breeding trials, in which the animals of both sexes had been reared on the fundamental tobacco seed diet, one male animal only failed to prove potent; the females were invariably able to produce offspring. These experiments were extended, with considerable success, to the production of a third generation of animals on the same diet.

It is obvious that the total protein of tobacco seed is of good biological quality. Although no feeding experiments on the isolated globulin have yet been attempted there is every reason to suppose that it resembles, with respect to the presence of the amino acids essential in nutrition, the proteins of other oil seeds for which data have been obtained.

5846

"Slow-Motion" Cinematographs of the Contraction of Single Cardiac Muscle Cells.

C. M. GOSS. (Introduced by S. R. Detwiler.)

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The material for this study was supplied by tissue cultures of embryonic heart in which the cardiac muscle had grown out and become differentiated. The ventricles of 16 day rat embryos were cut into fragments 1 to 2 mm. in diameter and cultivated in a medium composed of rat plasma and embryo juice. In the course of 2 to 4 weeks a new growth of muscle appeared among the fibroblasts, taking the form of anastomosing bands similar to adult cardiac muscle. This new growth was closely applied to the under surface of the cover glass so that single cells could be observed with the highest powers of the microscope. Cross striations could be seen in many of the muscle cells of both explant and new growth after one month. The striated appearance in the living cell was produced by the arrangement of small brick-shaped bodies, apparently mitochondrial in nature, into more or less regular longitudinal and transverse rows. Since this picture was somewhat different from that presented by muscle from an adult animal it seems likely that we were dealing with a transition stage in which the differentiation was incomplete. The presence of myofibrillæ with alternating dark and light bands and "membranes of Krause" in fixed preparations, however, indicated that the process was well advanced. Although the muscle in these cultures contracted spontaneously, its activity was intermittent, especially when exposed to room temperature for the making of photographs. This irregularity was overcome by substituting Ringer solution for the usual medium.

Since the intracellular movements were too rapid to be followed by direct observation through the microscope, an effect of slowing was accomplished by making "slow-motion" cinematographs. Exposures were taken at the rate of 32, 48 and 64 per second. The camera, a Bell and Howell 16 mm model 70D, without lenses, was mounted over a compound microscope. The lens system of the microscope consisted of a long-range substage condenser and a Zeiss apo. 120, n.a. 1.3 objective without an ocular.

The following observations were made:

1. Myofibrillæ were not visible in the living cells. The clear hya-

line matrix lying between the longitudinal rows of brick-shaped bodies mentioned above gave an appearance suggestive of fibrillæ but they were not interpreted as such. The only other structures visible in the living cells were occasional spherical droplets of neutral fat imbedded in the matrix.

2. The hyaline matrix or sarcoplasm was the active substance during contraction. The mitochondrial bodies appeared to be forced together in a direction parallel to the long axis of the fiber. The strips of sarcoplasm at their ends, corresponding to the light band of cross striation, became markedly thinner, at times being almost obliterated, while the strips of sarcoplasm at their sides, whose presence suggested longitudinal striation or myofibrillæ, became somewhat thickened. The shape of the blocks themselves did not change. In other words, the blocks were comparatively rigid structures suspended in a relatively fluid matrix and as they were passively moved closer together by the shortening of the cell as a whole, the matrix was forced from its position at the ends of the blocks to the spaces at their sides.

3. No reversal of striation was observed, but it must be remembered that these fibers may present a primitive type of striation and that the pictures used for study were not made with polarized light.

4. The contraction traveled with a wave-like motion from one end of the cell to the other. Occasionally twitches were observed that involved only a part of the cell, in which case the wave-like appearance could not always be identified.

5. The period of time occupied by the contractile phase was approximately 3 times as long as that occupied by relaxation.

6. Although the hyaline matrix or sarcoplasm was of a fluid consistency it had much of the elasticity of a gel. Fluidity was indicated by the rapidity of movement of the intracellular blocks. A gel-like elasticity was suggested, on the other hand, by the fact that the interior of the cell did not become quiet immediately after contraction but quivered and frequently set up small reflected waves in a direction opposite that of the contractions.

5847

Influence of Anesthesia upon Pancreatic Function.*

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When a dog with pancreatic fistula secreting continuously is subjected to ether anesthesia the spontaneous secretion is largely or entirely suppressed according to the animal's susceptibility to ether. Under these conditions the level of blood amylase rises. In determinations according to Wohlgemuth, the normal level of blood amylase varies between 66 and 300 units in different animals, but it is constant in the same animal. Following ether anesthesia there is a 75% to 150% increase above normal values, as observed in 4 fistula dogs and 3 normal animals. Sodium amytal has little effect on the rate of secretion and none on the amylase level in the blood. (2 fistula dogs and 1 normal.) From this and other data we conclude that continuous secretion cannot be observed directly in acute experiments under ether anesthesia, but evidence of the continuous secretory function remains in the rise of blood amylase. Mechanical blocking of the flow has the same effect.

These observations led to the following comparative study concerning the influence of ether, avertin and spinal anesthesia upon blood amylase in man. Blood samples were obtained for analysis before anesthesia was induced and at frequent intervals during the 24 hour period immediately afterward. Normally, the blood was found to contain 12.5 to 20 units of amylase. The patients were of both sexes and varied from 24 to 57 years in age. They underwent various types of operations, such as hernioplasty, reduction of a fracture or removal of diseased adnexa. Upper abdominal operations were not included in order to avoid the possible effect of handling the pancreas upon blood amylase.

The amounts of ether varied from 226 cc. to 360 cc. The dosage of avertin was 100 mg. per kilo of body weight. Spinal anesthesia was induced by the injection of 120 mg. of novocain into the subarachnoid space.

In 4 patients subjected to ether there was a definite rise in blood amylase, reaching its maximum within 3 to 8 hours after induction

* We are indebted to Dr. John F. Connors of the Harlem Hospital and Dr. Allan O. Whipple of the Presbyterian Hospital for allowing us to study the patients on their respective surgical services.

of the anesthesia. The increase was 2 to 3 times the original level. A return to normal limits occurred within 24 hours. On the other hand, in 4 patients subjected to avertin anesthesia (3 supplemented with ether) and in 2 patients with spinal anesthesia no change in blood amylase was detected. In one patient with avertin anesthesia supplemented by ether, a slight rise in amylase was noted at the end of 14 hours; in this case the operation was a very difficult and prolonged one and there was considerable post-operative shock.

Conclusions. Following ether in man, there is a definite increase in blood amylase which does not occur after avertin or spinal anesthesia. According to the results obtained in dogs with fistulas, this is due to the fact that ether causes a cessation of the transport of pancreatic juice to the intestine while pancreatic secretory activity continues, resulting in a temporary accumulation of amylase in the blood.

5848

Differences in Bactericidal Power of the Blood Within an Inbred Strain of Rats.

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During the course of experiments on the rôle of heredity in the resistance of rats to infection with *S. enteritidis,* some discrepancies from the uniform mortality rate to be expected in a highly inbred strain of animals were encountered.[1] An immunological study of the animals from this strain showed marked differences in the bactericidal power of the whole blood. Loeb and King[2] had previously found marked differences in reaction to tissue transplants in rats within each of the Wistar "A" and "B" strains.

The animals used in our experiments were descended from one pair of rats of the Wistar "A" strain, obtained in 1924 from Doctor Helen Dean King. These rats were then in the 48th generation of brother-sister matings. These brother-sister matings have been continued in our tests. All rats used have been shown not to excrete *S. enteritidis* in their feces and were carefully kept free from ex-

* Fellow of the National Research Council.

1 Irwin, M. R., *Genetics,* 1929, **14**, 337.

2 Loeb, Leo, and King, H. D., *Am. J. Path.*, 1927, **3**, 143.

posure to this infection. Blood taken from the heart of each animal was mixed with just sufficient sodium citrate to prevent clotting, and drawn into capillary pipettes previously coated with a thin layer of a saline dilution of an 18 hour culture of *S. enteritidis*, the method being that proposed by Heist and Solis-Cohen[3] for determining the pneumococcidal power of blood. After the open end of the pipettes had been sealed in the flame, they were placed in the 38° incubator for 4 hours. The contents of each pipette were then expelled into a Petri dish of fluid agar and incubated over night, then the number of colonies counted. Suitable control tubes were inoculated to demonstrate the viability of the organisms and to indicate the numbers of organisms present in each dilution. Duplicate tests, after time intervals of 10 days to 2 weeks, gave consistent results.

For classification the animals have been divided into 2 groups: a "high" group whose blood destroyed more than 250 organisms per cc., and a "low" group, whose blood destroyed less than this number. However, nearly all of those falling within the "low" group showed no bactericidal power whatever—in most cases there was marked growth of the organisms in the blood. On the contrary the blood of most of the "high" group destroyed many thousands of organisms per cc. The result of a survey of 21 individuals of the inbred strain showed 17 to fit into the "low" group and 4 into the "high" group. These differences were so marked that matings were made to determine whether hereditary differences might explain the departure from a uniform reaction. Matings could not be made, at that time, among members of the "high" group. The blood of the offspring of these matings was tested when the individuals had reached 2 months of age and gave the following results:

Matings	Low	High	Total
Low × low	11	0	11
High × low	12	6	18

There appears to be some basis for attributing the differences shown above to heritable influences. The probability that the 2 sets of progenies are of the same population is well outside the limit generally taken as significant ($P = 0.03$), from which it may be inferred that the type of mating was a determining factor in producing individuals of the "high" group. Such differences occurring in an inbred strain may be attributed either to a result of mutation, or as a result of the continuous mating of heterozygotes. A further

[3] Heist, G. D., Solis-Cohen, S., and Solis-Cohen, M., *J. Immunol.*, 1918, **3**, 261.

study of the appearance of such marked differences in reaction in inbred animals of uniform exposure to the infectious agent is being made from both the immunologic and genetic points of view.

5849

Plant Pigments in the Nutrition of the Chicken.*

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Karrer, Euler and Rydbom[1] have demonstrated that carotene has provitamin A properties for the chicken but that xanthophyll is without effect in this regard. Evidence has been presented by Capper[2] that carotene is converted to vitamin A by the chicken.

The present experiment was an attempt to confirm the results of Karrer and his associates and to extend the study to include chlorophyll. In addition a study was made of the uric acid[3] content of the blood, it having been observed in earlier studies that this constituent increases in amount in the vitamin A deficient condition. Newly-hatched White Wyandotte chicks were placed upon a vitamin A deficient ration: 52.5% white corn, 20% wheat middlings, 10% dried skim milk, 10% meat scrap, 5% wheat bran, 0.5% sodium chloride, 1% calcite flour and 1% steamed bone meal. Sufficient irradiated ergosterol was used to protect against leg weakness. On this ration retardation of growth begins between 3 and 4 weeks of age. The pigments were dissolved in ethyl laurate and dilutions made with cottonseed oil. The daily allowance of each pigment was 0.03 mg. by capsule. Supplementation with carotene (M.P. 170°-173°) began at 26 days, with xanthophyll (M.P. 173°), at 27 and with chlorophyll at 31 days. An immediate growth response took place when carotene was fed but the birds which received the xanthophyll and the chlorophyll not only failed to grow, but lost weight and died within 2 weeks. Their behavior was essentially that

* Journal Series Paper, N. J. Agricultural Experiment Station, Department of Agricultural Biochemistry.

[1] Karrer, P., v. Euler, H., and Rydbom, M., *Helv. Chim. Acta*, 1930, **8**, 1059.

[2] Capper, N. S., McKibbin, I. M. W., and Prentice, J. H., *Biochem. J.*, 1931, **25**, 265.

[3] Folin, O., *J. Biol. Chem.*, 1930, **86**, 179.

of the non-supplemented control group. Nine or more individuals were used in each group.

The uric acid of the whole blood was determined at 35 to 40 days of age. The average value for the carotene group was 3.7 mg. per 100 cc. of blood, whereas that for the xanthophyll group was 7.2 mg., and for the chlorophyll group 5.8 mg. The birds which subsisted on the unsupplemented white corn ration showed an average value of 9.8 mg., while those receiving the same ration but with the white corn replaced by yellow corn had a uric acid value of 3.3 mg., which is considered normal. Our observations with a number of young chicks show the normal uric acid value to be 4.00 mg. per 100 cc. of whole blood or less. In earlier work a high uric acid value was noted in birds deprived of food. It is not known whether the high values noted in vitamin A deficient chicks are due indirectly to the deficiency which is accompanied by a reduced intake of food or whether the vitamin A deficiency is directly responsible.

The results confirm those of Karrer, Euler and Rydbom with regard to carotene and xanthophyll. In addition chlorophyll has been shown to lack provitamin A properties.

5850

Quantitative Aspect of the Hypothetical Incorporation of Injected Antigen in Resulting Antibody. II. Experimental.

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The authors[1] have previously examined, from an arithmetical point of view, the Buchnerian hypothesis that antibody is a conjugate of body globulin and injected antigen. These speculations led us to undertake the following experimental investigation based upon the reactivity of artificial compound proteins in which the hapten chosen to confer the new specificity contains integrally an inorganic atom (As) for which a direct and very delicate chemical test is available. Two compounds, ovomucoid-diazo-arsanilic acid and casein-diazo-arsanilic acid, were prepared. The serum of a rabbit immunized against the first reacted with the second, showing the presence of an arsanilic-acid-specific antibody. The optimal precipitation ratio by volumes of antiserum to casein-diazo-arsanilic acid

[1] Hooker, S. B., and Boyd, W. C., *J. Immunol.*, 1931, **21**, 113.

was found to be about 150. Such a mixture is considered neutral—at least neither reagent is present in any large excess. It seems reasonable to suppose that within this zone, antibody and antigen enter respectively into the precipitate in the proportion of one molecule to one molecule, or one molecule to a small number of molecules. If it is assumed that the antibody is a globulin conjugate of the antigen, and contains a similar amount of arsenic (as would necessarily be true if there were no breaking up of the injected antigen), it can be shown from the amounts of arsenic and casein found in the casein-diazo-arsanilic acid solution, and the above ratio, that 10 cc. of antiserum should have contained about 20 mmg. (20×10^{-6} g.) of arsenic, an amount easily detectable. None was found. This would seem to cast doubt on the Buchnerian hypothesis.

In a recent paper Heidelberger and Kendall[2] similarly come to the conclusion that the injected antigen in one of their experiments must have split into at least two specifically reactive fragments, if the Buchnerian hypothesis be true.

However, from the amounts of arsenic and casein in the casein-diazo-arsanilic acid solution it is possible to arrive at a new estimate, namely, the number of diazo (hapten) groups in combination with one molecule of protein. For casein it was found to be 110, which in view of the roughness of the data may be considered a fair approximation to the theoretical value of 160. The questions now arise—with how many of these groups (haptens) must antibody react in order to produce precipitation, and how much is the injected antigen supposed to break up *in vivo* before entering into the specifically reactive hypothetical antibody-conjugate? The answers seem to be within reach of further experimental inquiry in which the authors are now engaged.

We are indebted to Mr. Saul Kamens for assistance in this work.

5851

Experiments on Gill Reduction in Neotonous Triturus viridescens.

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From the Department of Zoology, Mount Holyoke College.

In certain regions neotonous individuals of *Triturus viridescens* are found in the same ponds with metamorphosed ones. Their gills

[2] Heidelberger, M., and Kendall, F. E., *Science*, 1930, **72**, 252.

persist after the body fin has been lost and the sex organs are well developed.[1] The object of these experiments was to find out whether such gills could be affected by environmental changes. If this occurred it would indicate that the hereditary background of these newts was different from that of those that lose their gills early.[2] Attempts to reduce the gills were made (1) by forced residence in a dry environment, (2) by implants of anterior pituitary lobe taken from gill-less adults from another region and (3) by implants of thyroid gland from the same source.

Gilled newts from Woods Hole, 6 to 10 cm. in length, and typical gill-less adults from South Hadley collected in October and November were used. Experiments were carried on through the winter and spring. Among 500 Woods Hole newts, 75% had gills on each side containing circulating blood. Their sex organs were partially or completely developed. The thyroids of 6 gilled newts suggested a state of rather low activity.[3] A slightly less inactive condition obtained in thyroids of gill-less individuals from Woods Hole. On the other hand, the thyroids of South Hadley adults appeared active (compare adult *Ambystoma opacum,* Uhlenhuth[3]).

On October 14, 1930, 2 lots of Woods Hole newts with gills averaging 0.13 cm. in length were divided into 4 groups according to size. Twenty were kept in jars cushioned with moss occasionally sprinkled with water. Twenty others were kept in jars of water. Ten aquatic adults (South Hadley) were kept in moss-bedded jars and 10 in water. All were kept under the same conditions. Of the 20 gilled newts subjected to dry conditions, 10 were kept for 7 months or more. At that time the gills were shrunken but they expanded when moistened and circulating blood was visible within them.

In other experiments gilled newts kept in water were given bi-weekly grafts of anterior pituitary from donor adults (South Hadley) for 10 weeks. One set of controls was engrafted with intermediate and posterior lobes; another set was given no treatment. After the fifth anterior pituitary implant tail-fins appeared and the males developed characters typical of the breeding season. Within 20 days, 4 females laid eggs, continuing to deposit one to 8 at frequent intervals. Motile spermatozoa were found in the cloacae of the males. There was no noticeable change in the gills during the experiment nor for several weeks afterward.

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[1] Noble, G. K., *Am. Mus. Nov.*, 1929, No. 348.

[2] Van Swinderen, M., *Tydschr. Neder. Dierk. Vereen.*, 1925, **19**, 141.

[3] Uhlenhuth, E., *Roux' Arch. f. Entw. Mech.*, 1927, **109**, 609.

In the thyroid experiments, 2 thyroid glands from gill-less adults (South Hadley) were implanted bi-weekly into 20 gilled newts. No reduction of the gills was detected during the time that the animals survived, 10 to 27 days following the first transplant.

Throughout the experiments the gills appeared insensitive to pituitary and thyroid grafts although the condition of the sex organs, and the molting and emaciation indicated that the implants were potent in certain ways. However, during the winter and spring season here represented, the tempo of gill reduction was not accelerated by a dry environment nor by administration of endocrine glands.

5852

Homology of Prooestrous Bleeding in the Dog.

ROLAND K. MEYER* AND SEIICHI SAIKI.† (Introduced by G. W. Corner.)

From the Department of Anatomy, University of Rochester School of Medicine and Dentistry.

The prooestrous uterine bleeding of dogs and menstruation of primates have been considered by some physiologists to be very similar and to represent in 2 classes of mammals the result of similar causative factors. (Heape,[1] Marshall.[2])

We have obtained data from experiments conducted on dogs which we believe strongly indicate that the prooestrous bleeding of the dog is not homologous with the menstruation of primates. The evidence for this belief is as follows: (1) Ovariectomy of 5 dogs in the metoestrous phase did not result in external or profuse microscopical bleeding. In all 5 cases microscopical bleeding occurred as early as the 7th day and as late as the 16th day after ovariectomy. This bleeding was only of one or 2 days' duration. No external or microscopical bleeding was observed after ovariectomy of one immature dog and one dog in oestrus. Ovariectomy of monkeys results in external bleeding of about the same quantity as that seen at the normal menstrual period. (E. Allen[3]; Morrel *et al*[4]; Saiki, in

* National Research Council Fellow in the Biological Sciences.

† Rockefeller Travelling Fellow.

1 Heape, W., *Quart. J. Micr. Science*, 1900, **44**, 1.

2 Marshall, F. H. A., *Quart. J. Exp. Physiol.*, 1927, **17**, 205.

3 Allen, E., *Publ. Carnegie Inst. Washington*, 380. (Contrib. to Embryol., 19, 1.)

4 Morrell, J. A., Powers, H. H., Varley, J. R., and de Frates, J., *Endocrinol.*, 1930, **14**, 174.

press.) (2) Injection of alkaline extracts of the anterior lobe of the hypophysis‡ which contained the gonad stimulating hormones into 2 dogs with ovaries intact resulted in external bleeding during the period of injection. This bleeding stopped soon after the injections had been discontinued. In monkeys profuse microscopical or external bleeding does not occur during the injection period. A latent period of some days intervenes between the cessation of injection and the appearance of blood. (Hartman[5]; Saiki, in press.) (3) Control experiments in which castrated dogs were injected with quantities of the anterior lobe extracts sufficient to produce external bleeding in the intact animal did not result in profuse microscopical or external bleeding. In 2 of the 3 dogs injected in this experiment a few red cells were seen in the lavage on one or 2 days of the injection period. The presence of these cells we do not interpret as being significant in the consideration of the causation of the prooestrous bleeding. (4) Injection of oestrogenic substances (amniotin, theelin) in large amounts (1100-3000 r.u.) into 5 castrate dogs produced macroscopic external bleeding in all cases during the injection period. As observed in the case of administration of anterior lobe in the intact dog, external and microscopical bleeding stopped soon after the injections had been discontinued.

Administration of oestrogenic substances to monkeys does not produce a profuse microscopical or external bleeding during the period of injection. External bleeding is found to occur a few days after the injections have been discontinued. (E. Allen[3]; Morrell *et al*[4]; Saiki, in press.) (5) Biopsies of the uteri of dogs taken at the time of maximum bleeding (produced either by anterior lobe extracts in intact dogs or oestrous hormone in castrate dogs) did not show upon microscopical study any of the characteristic changes seen in the primate uterus at the time of menses. (Menses with or without a corpus luteum.) (E. Allen[3] and Corner.[6]) (Marshall and Jolly[7] state that epithelial degeneration does occur in the uterus of the dog during prooestrous bleeding. However, we have not seen definite alterations in the epithelium of the uterus of the dog at

‡ We are very grateful to Parke, Davis and Co. for furnishing us with the anterior lobe extracts and the theelin, and to E. R. Squibb and Sons for the amniotin.

[5] Hartman, C. G., Firor, W. M., and Geiling, E. M. K., *Am. J. Physiol.*, 1930, **95**, 662.

[6] Corner, G. W., *Publ. Carnegie Inst., Washington*, 332. (Contrib. to Embryol., 15, 73.)

[7] Marshall, F. H. A., and Jolly, W. A., *Phil. Trans. Royal Soc.*, London, 1905. 198 B, 99.

the time of bleeding which we are willing to call degenerative.) There is much extravasation of blood into the subepithelial tissue of the dog during the bleeding but no lacunae of blood are seen such as those found in the menstruating primate uterus. The stromal tissue in the areas of extravasation does not appear to be degenerated. These findings are in accord with the general opinion that stromal degeneration does not take place in the uterus of the dog at the time of the prooestrous bleeding.

These data demonstrate that the prooestrous bleeding of the dog is produced by direct or indirect action of the oestrous hormone on the uterus. We believe the mechanism by which this hormone produces its effect cannot be considered to be identical with that concerned in the production of uterine bleeding in primates.

5853

Rate of Beat Over Long Time Periods of Isolated Turtle Hearts Treated with Thyroxin.

E. NEWTON HARVEY AND COLIN MACRAE.

From the Physiological Laboratory, Princeton University.

The question arises whether the marked increase in rate of heart beat which develops in hyperthyroid and in thyroid fed animals will appear in isolated hearts treated with thyroxin if a sufficient time is allowed for the action of the hormone. Lewis and McEachern[1] have shown that hearts removed from thyroid fed, or thyroxin treated rabbits maintain a 50% increase in rate for 8 to 10 hours, and that normal excised rabbits' hearts show no immediate effect of thyroxin.

We have kept excised turtle hearts at 20°C. ± .02° for 60 hours. recording automatically the rate during this period by means of the Loomis chronograph.[2] The heart was tied by the tips of the auricles to a heart lever and suspended in a modified Ringer's solution, containing 0.1% glucose and phosphate buffer to pH = 7.3, oxygenated by pure O_2 gas. Addition of thyroxin*, dissolved in a minimal

[1] Lewis, J. K., and McEachern, D., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 504; *Bull. Johns Hopkins Hospital*, 1931, **48**, 228.

[2] Loomis, A. L., Harvey, E. N., and MacRae, C., *J. Gen. Physiol.*, 1930, **14**, 105.

* We take pleasure in expressing our thanks to E. R. Squibb and Son, who kindly supplied us with 100 mg. of crystalline thyroxin.

amount of alkali as a stock solution of 1:1000, does not change the pH of the solution. The details of transmitting the heart beat to the chronograph are described in a previous paper.[2]

The hearts beat about 10 times in 20 seconds at first, gradually slowing to 10 beats in 30 seconds in 2 hours when the thyroxin, $1:10^5$, is added. No immediate change in rate occurs, the heart gradually slowing, with some rhythmic variation in rate, over a period of 2 days, when the rate may be 10 beats in 80 seconds. The 4 hearts studied showed no tendency whatever to increase in rate and behaved in every way like normal hearts, which likewise continually slow over long time periods. We, therefore, conclude that thyroxin has no delayed direct action on the heart after excision from the body. Its action in the body must be secondary, possibly the production of a substance which directly affects heart muscle and whose effect persists for some time. Epinephrin and ephedrin affect these hearts in the normal rate-increasing manner.

5854

Colony Variation in Pathogenic Strains of Bacterium coli Induced by the Use of Dyes.

CARL O. LATHROP.

From the Department of Bacteriology, Medical School, University of Buffalo.

There is perhaps no other single criterion for the identification of the culture type that is so significant as colony appearance. It is regrettable, however, that in many investigations slight or no effort is made to proceed beyond recording the colony appearance. That this procedure is ill-advised is evident to every investigator who finds discrepancies in the characteristics of apparently established types.

In connection with studies on the pathogenicity of certain strains of *Bacterium coli,* note was made of the fact that some dyes tended to cause the appearance of clearly defined rough colonies with marked consistency in cultures that had been quite uniformly smooth for many generations. Use was made of a series of dyes to ascertain whether this was a constant phenomenon, perhaps associated with specific dye radicals, and there were found 4 of 25 common dyes which gave definite results.

Methylene blue (1-1000) yielded 60% roughs on the first trans-

fer, with no satisfactory increase toward the desired 100% on repeated transfer. Crystal violet (Grübler, prewar stock) yielded 70-80% roughs on the first transfer with some tendency to increase on repeated transfer but falling short of 100%. Neutral acriflavine (1-1000) produced no roughs but practically 100% intermediates. Acriviolet, a mixture of crystal violet and neutral acriflavine, averaged about 95% roughs on the first inoculation, while the second, or at most the third transfer, even of thoroughly stabilized smooths, yielded 100% dissociation into rough colony types. The base used in these dye experiments was plain beef extract 3% agar, with no enrichment, hence there was no conflict between elements tending toward roughness and smoothness. A later report will deal with the restoration of roughs to smoothness, with a discussion of the effect of certain enriching substances favoring smoothness.

After the identity of the variants obtained had been established as true roughs with the single exception occasionally noted with other organisms, that motility was still active, the usual virulence tests were done and with the standard intraperitoneal inoculation the smooth strains maintained their original virulence and the rough strains proved avirulent. However, when intravenous inoculation was employed, the rough strains, 12 in number, all caused death of the inoculated guinea pigs in 24 hours or less. On autopsy, the lungs were markedly congested with a typical lobar pneumonia, and the organisms were recovered in each instance from the lungs. The autopsy cultures of the rough strains recovered from the lungs showed about 5% reversion to smoothness, which corroborated earlier observations that blood, while unable alone to accomplish reversion of roughs to smoothness, nevertheless favored such change.

The animals injected intravenously with the homologous smooth strains succumbed a few hours later with septicemia and no lung involvement. This was so unexpected and so subversive of the idea that avirulent strains had been obtained in the process of conversion from the normal smooth to the atypical rough that it was very carefully checked, using rabbits as well as guinea pigs. In every animal used, the rough strains, intravenously injected, caused acute death, usually in less than 20 hours, while the smooth ancestor strains consistently caused septicemia, from which cases autopsy cultures yielded undifferentiated smooth strains. Cultural studies of the rough variants showed them to be much more active fermenters of carbohydrates than their smooth ancestors. Further study is being made on the significance and mechanism of the described rough strain pneumonias.

I used 60 guinea pigs and 24 rabbits: (1) 12 guinea pigs, original virulence tests, intraperitoneal inoculation; (2) 12 rough variants from original smooth strains, intraperitoneal inoculation; (3) 12 same as (2), intravenous inoculation; (4) 12 same as (3), rechecks; (5) 12 smooth cross-checks from original smooth strains, intravenous inoculation; (6) 12 rabbits, smooth cross-checks, intravenous inoculation; (7) 12 rabbits, rough variants from original smooth strains, intravenous inoculation..

5855

Hyperplasia of Thyroid and Exophthalmos from Treatment with Anterior Pituitary in Young Duck.*

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From the Department of Anatomy, College of Physicians and Surgeons, Columbia University.

It has been definitely shown that the normal structure of the thyroid gland is dependent upon an autacoid present in the anterior lobe of the pituitary gland. In amphibians its release at the time of metamorphosis stimulates the thyroid to excrete its content and induces the increased cellular activity characteristic of this period. In mammals Loeb and his coworkers[1, 2] and Aron[3, 4] showed that the injection of anterior pituitary extracts and emulsions produced also some characteristic changes in the thyroid suggestive of a higher activity. The follicular epithelium became higher, mitoses were present and colloid was excreted. The glands increased in size from 2 to 4 times and became more vascular. The metabolism of treated guinea pigs, as shown by Siebert and Smith[5] was markedly increased. As previous observations[6] had shown that young ducks were very sensitive to pituitary extracts and emulsions, it seemed

* Aided by a grant from the Special Research Fund of Columbia University to the Department of Anatomy.

† Fellow of the C.R.B. Educational Foundation.

1 Loeb, L., and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 860.

2 Loeb, L., Bassett, R. B., and Friedman, H., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 209.

3 Aron, M., *C. R. Soc. Biol.*, 1929, **102**, 682.

4 Aron, M., *Revue fr. Endocrin.*, 1930, **8**, 472.

5 Siebert, W. J., and Smith, R. S., *Am. J. Physiol.*, 1930, **95**, 396.

6 Schockaert, J., *Arch. intern. Pharm. Thérap.*, 1931, **41**, 23.

worth while to investigate the possibility of inducing in this species functional and structural changes indicative of hyperthyroidism.

Forty-two young ducks weighing between 300 and 500 gm. were used: 22 of these were treated with anterior pituitary suspension, Phyone, luteinizing hormone or Prolan, 9 were injected with spleen emulsion as controls, and 9 were left untreated. The fresh, centrifuged, saline suspension of beef anterior pituitary given in a daily dosage of about half a gland per animal induced a marked and rapid increase in the size of the thyroid lobes, which reached after one or 2 weeks of treatment, 3 to 8 times the size of those of untreated or spleen-injected controls. In one case a relative increase of 20 times was observed, and in another, the thyroids reached 60 times the weight of the control thyroids.

The shorter periods of treatment (1 to 7 days) produced little change in the size of the vesicles, but colloid disappeared and the epithelium became of a higher columnar type showing often pycnotic nuclei, desquamation and mitoses, a picture entirely similar to that of some acute types of Graves' disease.

In more prolonged treatment the vesicles enlarged, the epithelium became folded and formed hyperplastic buds in the vesicles. Some colloid, which was granular and stained faintly, appeared again. At three to five weeks the treated ducks developed gradually an exophthalmos particularly noticeable when viewed laterally and inferiorly. If treatment was stopped, the bulging of the eyeballs disappeared entirely in about one week. Under ether anesthesia the bulging promptly vanished to slowly appear again when the animal awakened. It was never observed in the control animals, untreated or injected with spleen or liver suspensions.

Chemical analysis‡ (Pregl's method) showed that the total iodine content of the glands had dropped considerably even after a short treatment, and reached a level of 1/20 the normal content, even when new colloid had been secreted after prolonged treatments. This colloid consequently must be considered as chemically different from the colloid normally present, from which it differs also by its granular structure and its faint staining.

Prolan and luteinizing hormone (from urine of pregnant women) were found to be inactive on the duck thyroid, histologically as well as chemically.

On the other hand, the fraction which contains the growth hormone (van Dyke and Wallen-Lawrence's Phyone) induced an in-

‡ The chemical data secured in collaboration with Professor G. L. Foster, will be published in a joint paper to appear in the *Journal of Biological Chemistry*.

crease in weight, histological changes and a marked drop in the iodine content, though to a lesser degree than the whole suspension.

5856

Precocious Development of Sexual Characters in the Fowl by Homeoplastic Hypophyseal Implants. I. The Male.*

L. V. DOMM. (Introduced by Frank R. Lillie.)

From the Whitman Laboratory of Experimental Zoology, University of Chicago.

A series of light brown Leghorn cockerels ranging from 22 to 86 days in age were given daily subcutaneous homeoplastic hypophyseal implants. In the first experiment the entire hypophysis was utilized while in subsequent tests only the anterior lobe was employed. The number of daily implants per bird varied from 19 to 28. The treated birds, as well as controls, were weighed and the head furnishings measured at regular intervals, at first daily, later on alternate days. All treated individuals remained active and in excellent condition throughout the experiments so that they could not be distinguished from controls in this respect. In fact treated males were consistently somewhat heavier than their respective controls when treatments were discontinued.

One of the effects, quite apparent after 4 to 5 days' treatment, was a stimulation of head furnishings. These became turgid and reddish in color and revealed noticeable growth. In the longer treatments there was a noticeable slackening in growth toward the close of the experiment while in some of these there was a cessation followed by a slight regression in size. Bird No. 167, 59 days old when the experiment began, received 23 daily implants. On the day the experiment started its comb measured 4.7 cm. in length and 2.6 cm. in height, while 15 days later it measured 7.5 cm. in length and 4.5 cm. in height, the maximum size attained. On the day following the last implantation, 24th day of the experiment, when the bird was killed, measurements of comb were 6.8 cm. in length and 4.0 cm. in height. The best control comb showed a gradual increase during this period from 4.5 cm. in length by 2.6 cm. in height to 5.8 cm. in length by 3.5 cm. in height. Precocious sexual behavior

* This investigation was supported in part by a grant from the committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. Frank R. Lillie.

was very evident in the older individuals treated which were known to crow and tread. No modifications were observed in either plumage or spurs.

Post-mortem examination revealed pronounced hypertrophy of testes in all treated individuals. These modifications can perhaps be best appreciated by concrete data on an average case. Bird No. 182, 39 days old when experiment began, received 19 daily implants and was killed on the day following the last implantation. The right testis measured 1.2 cm. in length and 0.7 cm. in width, while the left measured 1.4 cm. in length and 0.6 cm. in width. Measurements of the best developed control gonads were right 0.9 cm. in length by 0.3 cm. in width, left 0.9^{+} cm. in length by 0.3 cm. in width. Hypertrophy of *ductus deferens* was not pronounced. Macroscopically such organs as the thyroids, spleen and liver showed little if any modification. Thyroids were small and difficult to find in treated as well as control individuals.

Histological examination of testes revealed very significant modifications. Whereas controls showed juvenile tubules in various stages of development, the tubules of treated individuals in all cases were greatly advanced with the older cases showing complete spermatogenesis with an abundance of mature sperm. This condition normally would not have occurred until the birds were many weeks older. The amount of interstitial tissue in proportion to tubules seemed no greater in treated individuals than in controls. No histological difference could be detected between the thyroids of treated and control individuals.

Hypophyses were obtained from mature cocks, hens, and capons, though these were not used indiscriminately, care being taken to utilize the glands from a particular sexual type exclusively in any one experiment. There is some evidence indicating that the hypophysis of the capon is most effective, that of the hen least. The effects appeared to be similar whether the entire hypophysis or merely the anterior lobe was employed.

It is assumed that the gonad-stimulating substances of the introduced pituitary anterior lobe acted directly on the testes and these in turn are responsible for the precocious development of the other sexual characters.

Preliminary experiments in collaboration with Dr. H. B. Van Dyke using a purified gonad-stimulating hormone prepared from sheep pituitary glands yield even more pronounced effects in some respects.

5857

Precocious Development of Sexual Characters in the Fowl by Homeoplastic Hypophyseal Implants. II. The Female.*

L. V. DOMM. (Introduced by Frank R. Lillie.)

From the Whitman Laboratory of Experimental Zoology, University of Chicago.

In a series of experiments daily subcutaneous homeoplastic hypophyseal implants were made on juvenile light brown Leghorn females.[1] These ranged in age from 28 to 59 days respectively when experiments began. In only one experiment was the entire hypophysis employed, while in all others only the anterior lobe was utilized. The number of implants received in any one experiment varied from 19 to 28. Weights were taken and measurements of head furnishings made at regular intervals as in experiments on cockerels. Treated females remained in excellent condition and were not adversely affected throughout any of the experiments. In none of the experiments was there a significant difference in weight between treated and control. In some the treated individuals were a trifle heavier than their respective controls, in others the reverse was true. This was not the case with treated males which were usually significantly heavier than their controls.

The first and most obvious effect to be noted was the phenomenal growth of head furnishings. These, pale and small when experiments began, became reddish and turgid after 5 to 6 days' treatment and revealed steady growth. Bird No. 178 was 22 days old when experiment started. It received 28 daily implants and was killed on the day following the last treatment. Its comb measured 1.3 cm. in length and 0.4 cm. in height when the experiment began, while on the day following the final implant it measured 4.0 cm. in length and 2.1 cm. in height, a creditable size for a pullet 4 months of age. The comb was quite stout, firm, and erect, resembling much more a male comb of similar size than the flabby, loppy comb of similar dimensions on an older female. The comb of the control was of the same size as that of the treated individual when the experiment commenced but measured only 1.8 cm. in length and 0.6 cm. in height at the conclusion of the experiment, a rather significant difference. Normally the comb of the female reveals no appreciable

* This investigation was supported in part by a grant from the committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. Frank R. Lillie.

[1] For similar experiments with males see Domm, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 308.

growth until it is several months older. No changes were noticed in either plumage, spurs, or behavior.

Necropsy revealed no signs of ovulation. The general appearance of treated ovaries was similar to that of controls though they were somewhat larger. The oviducts, however, showed a remarkable hypertrophy, having become large and convoluted, a phenomenon which normally precedes ovulation. Even the cloacal stumps of the rudimentary right oviduct were considerably larger in treated than controls. Right rudimentary gonads appeared somewhat larger, while rudimentary Wolffian ducts were notably larger in treated individuals. As in the male, thyroids were apparently not affected. Liver and spleen, as in the male, showed little if any apparent effect.

Histologically ovaries from treated individuals appeared unmodified. Follicles in such ovaries were evidently no more numerous than in controls and showed approximately the same degree of development. Sections of the oviduct in treated individuals, however, revealed a striking difference indicating a favorable action on the ovary. In experiment H 5, to which female No. 178 (see above) belonged, control oviducts had a diameter of less than 1 mm. and revealed low mucous folds without tubular glands or evident muscle layer, while experimental oviducts had a diameter of 4 mm. and revealed high mucous folds extending into lumen, many well-developed tubular glands and conspicuous peripheral muscle layer. It was difficult to detect any histological change in sections of rudimentary right gonads though experimental gonads appeared somewhat larger than controls. Sections of rudimentary Wolffian ducts examined revealed a distinctly larger lumen, a somewhat deeper columnar epithelium, and a much more conspicuous muscle layer in treated individuals. A preliminary study of thyroid sections revealed no modifications.

The experiments were conducted precisely as those on cockerels, utilizing hypophyses from similar sexual types. Some of them were conducted simultaneously on males and females of the same age. Results appeared to be similar whether we employed the entire hypophysis or merely the anterior lobe. The hypophysis of the capon appeared to be most effective, that of the hen least.

It is assumed that the gonad-stimulating hormone, liberated by the hypophyseal implants, acted directly on the gonad tissues of the host producing a precocious endocrine, rather than gametogenetic, functioning, which in turn is responsible for the precocious development of other sexual characters. It is further assumed that the male hormone liberated by the stimulated medullary tissue is respon-

sible for the growth of head furnishings and rudimentary Wolffian ducts while the female hormone liberated by the stimulated cortical tissue is responsible for development of the oviduct.

Preliminary experiments in collaboration with Dr. H. B. Van Dyke using a purified gonad-stimulating hormone prepared from sheep pituitary glands yield even more pronounced effects in some respects.

5858

Influence of (H^+) Concentration on the Anesthetic Value of a Series of General and Local Anesthetics and Hypnotics.

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From the Lilly Research Laboratories, Marine Biological Laboratory, Woods Hole, Massachusetts.

The purpose of this investigation was to determine whether any differences were to be observed in the relative anesthetic effects over widely different pH ranges, comparing a series of general anesthetics with a series of local anesthetics and a series of hypnotics. Alcohols such as allyl and isoamyl, and also general anesthetics such as chloroform, ether, chloretone, etc., do not form salts and consequently their distribution between oil and water is not markedly changed by a change in the (H^+) concentration of the water phase.

Local anesthetics like neothesin, cocaine and butyn are lipo-soluble organic bases with relatively low solubility as free bases in water, but in the presence of acids they form water-soluble salts. Consequently the oil-water distribution coefficient shifts from a high oil-solubility and low water-solubility on the basic side, to a low oil-solubility and high water-solubility on the acid side.

In the case of the barbituric acid hypnotics exactly the reverse relation is found. The barbituric acids are lipo-soluble organic acids possessing very low water-solubility but on addition of alkali to the water phase water-soluble salts of the barbituric acids are formed. Consequently the distribution coefficient shifts from a high oil-low water solubility on the acid side, to a low oil-high water solubility on the basic side.

It follows from the above that if the induction of anesthesia is in any measure dependent upon the lipoid-water distribution coefficient of the anesthetic a shift from the acid or neutral to the basic side should exert little or no effect on the alcohols and general anesthetics, should increase the effect of bases like cocaine and decrease the effect of pseudo acids of the barbituric acid type.

The experiments to be reported in this experiment were conducted on arenicola larvae. The gelatinous strings containing arenicola eggs were cut into small pieces and kept under sea water in the laboratory. The larvae hatched in 24 to 48 hours and being heliotropic accumulated near the surface at the point most exposed to light. The mass of larvae was collected and added in 0.2 cc. amounts to dishes containing a given concentration of anesthetic in 10 cc. of sea water adjusted to the desired pH. This semi-quantitative procedure has given consistent results during 3 seasons at Woods Hole.

TABLE I.
Anesthetic Down Doses for Arenicola Larvae.

		cc. per 100 cc.	
	pH 7.0	8.0	9.0
Iso-Propyl Alcohol	2.5	2.5	2.5
Propyl Alcohol	0.5	0.5	0.5
Allyl Alcohol	0.25	0.25	0.25
Iso-Amyl Alcohol	0.1	0.1	0.1
Chloroform	0.012	0.012	0.025
Chloretone	0.025	0.025	0.025
	Local Anesthetics		
		gm. per 100 cc.	
Cocaine	0.01	0.005	0.0025
Procaine	0.002	0.001	0.0005
Neothesin	0.001	0.0005	0.0005
Butyn	0.001	0.00025	0.00025
	Hypnotics		
Barbituric Acids		gm. per 100 cc.	
Iso-Amyl Ethyl	0.006	0.025	0.05
Di-ethyl Carbinyl Ethyl	0.006	0.012	0.05
Normal Amyl Ethyl	0.006	0.012	0.05
Propyl Methyl Carbinyl Ethyl	0.003	0.006	0.012

Table I gives the minimum concentration of anesthetic expressed in the first group in cc., in the second and third groups in grams per hundred cubic centimeters required to render the larvae immotile within a period of 5 minutes. Even after exposure to the solutions in question for a period of an hour the larvae recover their full motility when transferred to a large excess of sea water. Variations in (H^+) concentration cause no variation in the anesthetic effect exerted by the first group of alcohols and general anesthetics. A shift toward the alkaline side from pH 7 to pH 9 causes an increase ranging from 2 to 1 to 4 to 1 in the anesthetic effect exerted by a series of local anesthetics. The same shift from pH 7 to pH 9 causes a diminution ranging from 4 to 1 to 8 to 1 in the anesthetic effect exerted by a series of barbituric acids.

The above data harmonize with the conception that the induction of anesthesia is in some measure dependent upon the lipoid water distribution coefficient of the anesthetic in the cell and its immediate environment.

5859

Ultraviolet Radiation Needed to Cure Rickets with Respect to Area of Skin Exposed.*

ARTHUR KNUDSON.

From the Department of Biochemistry, Medical Department of Union University, Albany Medical College.

Information upon the amount of radiation needed to cure rickets with respect to the area exposed is very meagre. Maughan and Dye[1] have shown that in chickens only a small amount of radiation is necessary to cure rickets and that the area covered by the feathers receives very little or perhaps none of the beneficial rays.

Eighteen rats at 4 weeks of age were put upon the Steenbock rachitic diet 2965 and after 21 days, having developed a severe degree of rickets, were ready for treatment. Treatment was continued for 21 days and degree of healing was judged by radiographic examination. As a source of ultraviolet radiation the General Electric Sunlamp with the Type S1 bulb was used. Previous studies had shown that exposure of the whole rat 30 minutes per day to the G. E. Sunlamp at 3 feet brought about complete healing of rickets in 3 weeks' time.

It was found that very little radiation is effective through the hair of the rat as an exposure of 2 square inches of unshaved back of the rat for 40 minutes daily produced only a beginning healing. Irradiation of the skin of the rat is much more effective as an exposure of one-fourth square inch of shaved back of rat for 20 minutes daily at 3 feet brought about complete healing of rickets in 3 weeks. Irradiation of this area of the skin produces greater healing of rickets than exposure of the whole animal for the same length of time. According to recent work of Diack[2] on the surface area of rats, this area of one-fourth square inch amounts to about one-eightieth of total surface area. It is surprising that such a small fraction of the total surface area of the rat can absorb sufficient of the rays to heal rickets. It is also surprising to note that the total amount of ultraviolet energy below 3200 A° needed to cure rickets in the rats is not more than 1.2 small calories.

In another series of experiments with 20 rats, it was found that

* This work was aided by funds from the General Electric Company, Schenectady, N. Y.

[1] Maughan, G. H., and Dye, J. A., *J. Opt. Soc. Amer.*, 1930, **20**, 279.

[2] Diack, S. L., *J. Nutrition*, 1930, **3**, 289.

the amount of radiation needed to cure rickets is directly proportional to the area of skin exposed. Thus an exposure of one-fourth square inch of skin for 20 minutes daily heals rickets completely in 3 weeks and similar results were obtained with an exposure of one square inch for 5 minutes, 2 square inches for 2.5 minutes or one-eighth square inch for 40 minutes daily.

In a third series of experiments with 14 rats it was noted that the total irradiation which was found by daily exposure to produce complete healing can be given in as little as 2 exposures 10 days apart.

These experiments emphasize how very little ultraviolet radiation is needed to cure or prevent rickets. Assuming that a similar relationship holds for humans as has been shown in the case of the rat, it is apparent that much smaller amounts of ultraviolet radiation will be effective in preventing or curing rickets than has hitherto been appreciated.

5860

Heparin in Blood Calcium Analyses.

G. WALTERMANN HOLT. (Introduced by Esther M. Greisheimer.)

From the Department of Physiology, University of Minnesota.

Many investigators have assumed that the calcium of heparin is not available for oxalate precipitation, as performed in the Clark-Collip[1] modification of the Kramer-Tisdall[2] method. Interest in this question was aroused by the difference of opinion of various workers regarding the concentration of calcium in serum and in heparin plasma. Cantarow,[3] in a series of 100 determinations, using heparin as an anticoagulant, found that the calcium level was 0.5 mg. to 1.0 mg. less in each 100 cc. of plasma than in the corresponding serum. Greisheimer and Arnold,[4] and Loucks and Scott[5] have published data on this question.

In the present study several different samples of heparin were used. Heparin was added to redistilled water, to calcium chloride solutions, to plasma, and to serum. These heparinized solutions,

[1] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

[2] Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **47**, 475.

[3] Cantarow, A., Calcium metabolism and calcium therapy, 1931, 36.

[4] Greisheimer, Esther M., and Arnold, A. W., *Am. Rev. Tuberc.*, 1926, **14**, 479.

[5] Loucks, M. M., and Scott, F. H., *Am. J. Physiol.*, 1929, **91**, 27.

with corresponding non-heparinized solutions as controls, were analyzed for calcium by the Clark-Collip modification of the Kramer-Tisdall method.

Dogs' blood served as the source of plasma and serum. All reagents were tested and found to be calcium-free. A microburet whose outlet was ground and fitted with a hypodermic needle was used for titration.

The recovery on calcium chloride solutions was 99.64%. Ten determinations on redistilled water containing 100 mg. % of heparin gave a mean calcium value of 1.170 mg. %. Twenty-seven determinations on calcium chloride solutions containing 10 mg. of calcium in each 100 cc. and on calcium chloride solutions containing, in addition, 100 mg. % of heparin, gave mean values of 9.964 and 11.134 mg. % of calcium, respectively, the difference being 1.170. Control serum and heparinized serum gave means of 10.503 and 11.969 mg. % of calcium, respectively, in a series of 52. The difference is 1.466 mg. % of calcium. Fifty determinations on serum and corresponding heparinized plasma gave calcium values of 10.516 and 11.683 mg. %, the difference being 1.167 mg. in favor of the plasma.

It is concluded that calcium in heparin is precipitable as oxalate.

5861

Platelet Count After Splenectomy and Other Operations.

L. C. FISHER. (Introduced by H. A. Reimann.)

From the Department of Medicine, University of Minnesota.

Steiner and Gunn[1] demonstrated that splenectomy in normal rabbits was followed by a constant increase in the number of circulating blood platelets. Other operations on rabbits, involving a similar degree of trauma, were also followed by an increase of platelets, which did not differ in time of occurrence, degree or duration from the increase observed after splenectomy. They believed that the degree of rise depended upon the amount of trauma sustained during operation.

These results, though applicable to the rabbit, do not apply to man. It is generally agreed that in humans, the marked increase in

[1] Steiner, P. E., and Gunn, F. D., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1088.

the number of platelets after splenectomy is due to the removal of the spleen and not to other surgical measures involved.[2] Evans,[3] and many other observers before him, demonstrated an abrupt rise in the number of platelets to a high level, beginning within a few hours after splenectomy. Others[4, 5] found that platelets often increased after other operations, but seldom, if ever, to such high levels as after splenectomy. The increase in number, furthermore, was delayed until about the sixth day after operation.

The following observations were made to emphasize the differences in the immediate response of platelets after splenectomy and after other surgical procedures in the human. An accurate method, elsewhere described,[6] was used for the counting of platelets. Counts were made before, and several times after operation in each case. The results are plotted in Fig. 1.

Cases 1 and 2 suffered from cirrhosis of the liver. Splenectomy was performed in both because of enlargement of the organ and for the relief of ascites. In both cases, the platelets beforehand were only moderately diminished. In both cases, the number of platelets increased nearly 50% within 2 hours after splenectomy

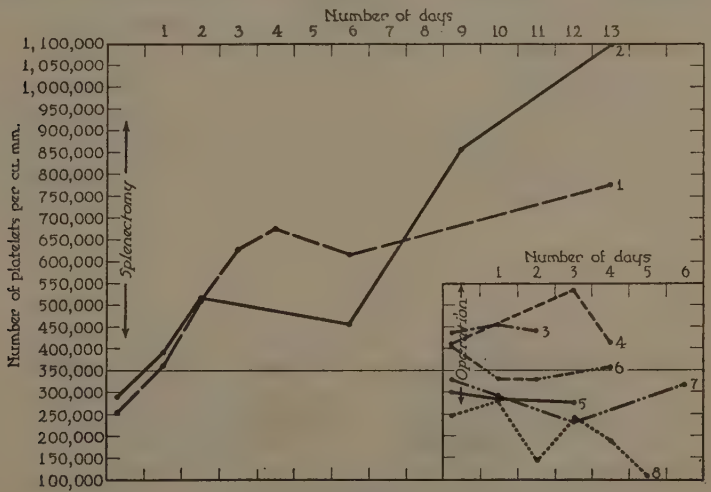

FIG. 1.

[2] MacKay, W., *Quart. J. Med.*, 1931, **95**, 285.

[3] Evans, W. H., *J. Path. and Bact.*, 1928, **31**, 815.

[4] Aynaud, M., *Comp. Rend. de la Soc. de Biol. Paris*, 1913, **74**, 373.

[5] Dawbarn, R. Y., Earlam, F., and Evans, W. H., *J. Path. and Bact.*, 1928, **31**, 833.

[6] Reimann, H. A., *J. Exp. Med.*, 1924, **40**, 553.

and continued to increase rapidly until the maximum number was reached on the thirteenth day in Case 2. These curves agree with the results obtained by practically all who have made similar observations.

Cases 3 to 8 were operated upon for other purposes. The record of the platelet counts in these cases is also illustrated in Fig. 1. It is evident that the striking increase which is observed after splenectomy does not occur. In fact, in 4 cases, the platelets diminished, as they do after many other procedures and conditions.[2] A decrease is noted in case 5 (excision of cervical polyp and herniorraphy), case 6 (herniorraphy), case 7 (herniorraphy) and in case 8 (hydrosalpinx, excision of cystic ovary). It is of course possible, if further observations had been made in these cases, that the platelets would have been found increased after the sixth day as in cases observed elsewhere.[5]

A slight, transient increase in the number of platelets occurred in case 3 (cholecystoduodenostomy) and in case 4 (posterior gastroenterostomy after cauterization and eversion of a peptic ulcer).

Conclusion. Increase in the number of platelets after splenectomy or other operations, according to certain observers,[1] appear to be rather constant in rabbits. The behavior of platelets after splenectomy in humans is very different from that after other operations. After splenectomy there is usually an abrupt rise to a high level beginning immediately after operation. After other surgical procedures the variations are inconstant. The number of platelets may decrease or increase.

5862

Experimental Edema Produced by Plasma Protein Depletion.

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(With the technical assistance of Augusta B. McCoord.)

From the Departments of Physiology and Pediatrics, University of Rochester School of Medicine and Dentistry.

A study was made of the water and chloride metabolism of dogs during the development of low serum protein edema. The sites of deposition of water and chloride were determined by analyses of tissues obtained at autopsy.

Dogs were fed a diet of known amount and composition. After a control period of one to 2 weeks, blood was removed every day for 1 to 7 days, was deprived of its plasma, and the washed cells suspended in calcium-free Locke's solution were returned to the animal. Blood and urine analyses were performed during both the control and experimental periods.

At suitable junctures in the experimental periods, the dogs were sacrificed by carbon monoxide gas and immediately autopsied. Tissues were removed and analyzed for water and chloride content. The values found on 7 dogs were compared with the average values of tissues taken from 3 dogs that had been subjected to various control procedures, and sacrificed and autopsied exactly as the hypoproteinemic animals.

Edema was found to occur consistently when the serum protein concentration was below 4.0%. The occurrence of the edema was associated with retention of sodium chloride and water, and the retained chloride and water were recovered with considerable accuracy in the tissues analyzed. Skin stored most of the water while most of the retained chloride was found in muscle. The retained water and chloride constituted an isotonic concentration in the skin and a hypertonic concentration in muscle.

Upon the basis of the data obtained, it is concluded that the edema which occurs in dogs rendered hypoproteinemic by plasmapheresis is a sodium chloride edema, the development of which is facilitated by the presence of a low serum protein concentration and exaggerated by increasing the sodium chloride intake and water intake of the animals.

5863

Shwartzman Phenomenon in the Rabbit Stomach.

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The Arthus phenomenon was first observed as a skin reaction and subsequently shown to occur in various internal situations with interesting and valuable results.[1] The Shwartzman phenomenon has been studied as a skin reaction in great detail,[2] but little attention

[1] Nordmann, M., *Physiol. Rev.*, 1931, **11**, 41.

[2] Shwartzman, G., *Klin. Wchnschr.*, 1930, **9**, 1925, 1974; *J. Exp. Med.*, 1931, **54**, 711.

has been given to the finer features of the lesion produced or to its manifestations in internal viscera. The observation of Frisch[3] that the "reacting factor" may be given intraperitoneally gives no information as to the occurrence of the phenomenon in internal viscera.

The stomach was selected as the organ first to be studied, because of its accessibility, the fact that control areas can readily be obtained and thin sections easily prepared for detailed microscopic study. Barium sulphate starch mixture was applied as a depilatory 24 hours before operation, which was performed aseptically under ether anesthesia, and the stomach exposed by high left rectus incision. The concentrated toxic filtrate of *B. coli* was prepared according to the method of Ecker and Rimington[4] and was shown to be sterile before use. The injection into the stomach wall of 0.2 cc. concentrated filtrate was into the submucosa, except in 1 instance where it was into the muscularis. Intracutaneous injection of 0.2 cc. concentrated filtrate was given in the lower abdominal region. Of 16 rabbits so treated, 12 were given 2.5 cc. concentrated filtrate intravenously 24 hours after the local injection. The animals were killed by fracture of the neck at periods of 3½, 5(2), 12, 24, 25(2), 72(2) hours, and 7 days (2) after intravenous injection. The 4 control animals, with local but no intravenous injections were killed at 7 and 24 hours and 7 days (2) after the local injection. After careful gross examination of stomach and skin, blocks were fixed in Zenker's fluid, embedded in paraffin and sections stained with hematoxylin and eosin.

It was found that the Shwartzman phenomenon occurs in the stomach wall and that the severity of the reaction is in general parallel to that of the skin. The peak of the reaction in the skin is reached in 4 to 5 hours after intravenous injection but in the stomach, the peak of reaction is at about 24 hours. The local injection into the stomach produces an exudative inflammation, which after the intravenous injection becomes more severe and shows hemorrhage. The hemorrhage occurs only in the animals with both injections, but in the stomach is not so severe as in the skin. The inflammation goes through organization and cicatrization. No gastric ulcers were observed.

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[3] Frisch, I. A., *Arch. Int. Med.*, 1930, **46**, 410.

[4] Ecker, E. E., and Rimington, C., *J. Hyg.*, 1927, **27**, 44.

5864

The Shwartzman Phenomenon in the Knee Joints of Rabbits.

ALAN RICHARDS MORITZ AND JOHN D. MORLEY.
(Introduced by Howard T. Karsner.)

From the Institute of Pathology, Western Reserve University, Cleveland, Ohio.

The Shwartzman phenomenon affords a new experimental method for the study of the local reactivity of joints to bacterial products.

The bacterial filtrates employed in this investigation were (1) from *B. coli* prepared by the method described by Ecker and Rimington[1] and (2) from *B. typhosus* supplied us through the kindness of Dr. Gregory Shwartzman.

The preparatory factor was injected lateral to and below the patella with the knee flexed, in amounts varying from 0.01 to 0.40 cc. of filtrate. Simultaneous injections were made into the skin of most animals. The intravenous injection of the 'reacting factor' was given from 20 to 30 hours later and animals were sacrificed from 1 to 192 hours after receiving the reacting factor in amounts usually of 1 cc. per kilogram body weight.

Seven control animals received only the 'preparatory factor' and were sacrificed at intervals corresponding to those of the experimental series.

The Shwartzman phenomenon was elicited in the synovial membranes of the knee joints in 6 of 11 rabbits tested. The reaction occurred with less frequency and severity in the joints than in the skin. Attempts to elicit the reaction in pleural and peritoneal cavities were negative. In rabbits sensitized simultaneously in knee joint and skin a severe skin reaction was not necessarily associated with a severe synovitis and vice versa.

The criteria employed for the recognition of the reaction were histologic and based on the presence of endovascular damage, followed by thrombosis and necrosis of the vessel with exudation of leucocytes and hemorrhage.

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[1] Ecker, E. E., and Rimington, C., *J. Hygiene*, 1927, **27**, 44.
[2] Shwartzman, G., *J. Exp. Med.*, 1928, **48**, 247.

5865

Effect of Ultraviolet Rays on Pharmacological Potency of Digitalis.

DAVID I. MACHT.

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Digitalis varies markedly in its pharmacological activity, even the alcoholic extracts deteriorating often within a comparatively short time. In view of the extreme differences of opinion held by pharmacologists and pharmacists in regard to the effect of ultraviolet rays on digitalis, the present investigation was undertaken. A standard tincture was kept in the ice-chest in the dark. Samples of this were irradiated with an Alpine Sun lamp, and with a water-cooled Kromayer lamp with and without various filters, and the pharmacological activity of the tinctures after assay was determined by 4 different methods: (1) the official one-hour frog method,[1] (2) the cat method of Hatcher and Brodie,[2] (3) the phytopharmacological method of Macht and Krantz,[3] and (4) the goldfish method of Pittenger and Vanderkleed.[4] The great majority of animal experiments were performed on cats because, in the author's experience, that is the most reliable method for assaying digitalis. About 100 cat experiments were performed in connection with the present investigation. Each sample or fraction of digitalis tincture was tested on at least 3 cats and in cases where the readings obtained showed a wide divergence, as many as 8 cats were sometimes used to obtain the average minimal lethal dose. The phytopharmacological method was found to be particularly adapted to comparative study of various samples of digitalis simultaneously and under identical conditions of light, temperature, barometric pressure, etc. At least one hundred sets of such experiments were performed and each control, as well as each sample of digitalis tincture, was tested on 8 or 10 healthy seedlings of *Lupinus albus*. The frog and goldfish methods were used only to corroborate the results obtained by the cat and phytopharmacological experiments. Each sample was tested on at least 10 goldfish and 12 frogs.

It was found that irradiation with ultraviolet rays of tinctures

[1] United States Pharmacopoeia. Tenth Revision (Tincture Digitalis, Assay), 1926.

[2] Hatcher and Brodie, *Am. J. Pharmacy*, 1910, **82**, 360.

[3] Macht and Krantz, *J. Pharm. and Exp. Therap.*, 1927, **31**, 11.

[4] Pittenger and Vanderkleed, *J. Am. Phar. Assn.*, 1925, **24**, 427.

exposed to the mercury vapor lamp in the quartz tubes produced deterioration of the tincture within ten minutes. Prolonging the irradiation produced greater deterioration up to a certain point, usually up to one and a half hours of exposure. After this point has been reached further irradiation for a limited period of time produces photochemical changes resulting in a greater toxicity or "potency" of the drug as tested by the methods mentioned above. Still further irradiation, for a period longer than two and a half hours, produces a second and more marked deterioration of the tincture. By the use of various filters it has been found that the longer ultraviolet rays produce little or no change in the tincture while the shorter rays produce even more rapidly the sequence of events noted above. This is particularly true when a gas filter, consisting of a mixture of chlorine and bromine and transmitting waves of 2400 to 2800$\mu\mu$, was used in conjunction with a Kromayer lamp. Specimens of digitalis tincture irradiated through such a filter first show a deterioration in potency but after exposure for over an hour the tincture begins to get more toxic or "potent". It is evident that exposure to ultraviolet rays produces progressive photochemical changes. There is also a possibility that several chemical reactions may be taking place simultaneously, these being produced by rays of different wave length. This is to be determined by study of individual rays with a monochromator.

The effects produced by x-rays and radium were also studied by the author, and it was found that exposure to both x-rays and radium emanations, as well as radium itself, produced marked deterioration of the tincture, as shown by the weaker action of digitalis on animal preparations. The phytopharmacological properties of such a tincture, however, are quite different from those of another exposed to ordinary ultraviolet rays. X-rays and radium render the digitalis tincture very much more toxic for plant tissues, while exposure to the ultraviolet rays of a mercury lamp makes it weaker for the same kind of plants.

5866

Comparative Pharmacology of Stomach Washings from Pernicious Anemia and Essential Achylias.

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The senior author has demonstrated repeatedly the presence of a specific toxin in the blood of pernicious anemia, which is not found in the blood serum of any other form of anemia, and is therefore of diagnostic interest as well as of value in following the results of various therapeutic procedures.[1] Later studies by Macht have revealed that a similar specific phytotoxic reaction is exhibited by spinal fluid from pernicious anemia patients, as compared with normal human spinal fluid.[2] This was found to be of value in differentiating neurological complications of pernicious anemia from similar cases of different etiology. Employing Macht's phytopharmacological methods, the authors made a systematic study of 125 patients in respect to toxicity of their stomach washings for the growth of living seedlings of *Lupinus albus*. A uniform procedure or technique was employed. A patient's stomach was washed in the morning, before food was taken, with 100 cc. of distilled water. The hydrogen ion concentration of the pumped-out fluid was then determined and the phytotoxic index was obtained. A 2% solution of the "washing" in each case was made in a standard plant-physiological solution and the growth of the straight, well-defined roots of *Lupinus albus* seedlings in the dark at a temperature of 20°C. for 24 hours was carefully measured. A comparison of these with control seedlings, grown under exactly the same conditions, was then made, and the effect of the washings, expressed as a phytotoxic index or percentage of growth in terms of the normal controls, was determined.

The average phytotoxic index obtained from stomach washings of 100 normal patients was found to be 86%. The lowest reading was 86%, while the highest was 100%. Stomach washings from cases of carcinoma showed no difference in toxicity as compared with normal subjects. The most interesting results, however, were found in cases of pernicious anemia and essential achylia, respectively. Phytopharmacological examination of the stomach washings for 9

[1] Macht, *Science*, 1930, **71**, 302.

[2] Macht, *Am. J. Physiol.*, 1930, **93**.

cases of pernicious anemia gave 60, 52, 47, 63, 55, 67, 63, 63, 62%, with an average phytotoxic index of 59%, while the phytotoxic index obtained from 8 cases of essential achylia gave 82, 95, 83, 82, 79, 82, 88, 90%, with an average index of 85%. All the 8 cases of essential achylias were of a very severe type, that is to say, each failed to respond with acid secretion to injections of histamine. The difference in toxicity in the 2 sets of cases bears no relation whatever to the hydrogen ion concentration of the solutions used as these were practically the same after the dilutions in physiological saline had been prepared. This difference in the readings between the achylias of pernicious anemia and those of idiopathic or essential type promises to be of diagnostic value. The toxicity of stomach washings from pernicious anemia cases is furthermore of interest in relation to various theories concerning the etiology of that baffling disease.

5867

Growth and Differentiation of Rat Embryos on the Chorioallantoic Membrane of the Chick.

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From the Osborn Zoological Laboratory, Yale University.

The experiments previously reported[1] have shown that rat embryos possess the potency to undergo a development of their tissues when implanted in strange surroundings. In order to test further the capacity of the embryonic parts for differentiation, transplantations of 8 and 9 day rat embryos have been made to the chorioallantoic membrane of the chick. This experiment was first performed by Hiraiwa[2] using rat embryos of a considerably older stage of development. He found that there was a considerable degree of self-differentiation in this form.

Up to the present about 200 such transplantations have been made; the eggs have been incubated from 7 to 9 days after transplantation (9 day hosts) and the grafts studied. In many cases the transplant causes only a minor reaction which is indicated by a slight thickening of the membrane on the site of the operation and a slight increase in the vascular field. This is the so-called

[1] Nicholas, J. S., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 188.

[2] Hiraiwa, Yoshi Kuni, *J. Exp. Zool.*, 1927, **49**, 441.

membrane reaction. In some cases a distinct nodule of tissue is found which upon section shows degenerating embryonic tissue. In these cases there are no embryonic parts except isolated tissue fragments.

In the positive series, containing embryonic parts, there are two groups: (1) those in which there is rapidly differentiating tissue and (2) those in which the tissues have developed exceedingly well but have undergone subsequent degeneration. So far we have had the opportunity to study the sectional material from 6 of these cases. Of these only one shows tissues undergoing differentiation at a rapid rate with a negligible amount of degeneration; 4 cases show the mixed type in which some of the tissues are undergoing differentiation and some are degenerating, while the sixth case shows practically complete embryonic degeneration.

The most favorable case shows not only that the tissues are capable of undergoing differentiation but that combinations of tissues are also possible in embryos developed under these conditions. Perfectly formed ear vesicles with nervous and membranous constituents are found together with heart, liver, gut, nervous tissue, muscle, and the supporting tissues.

At the stages used for the grafts, there is little tissue differentiation and no organ formation. A 9 day embryo is in the open medullary plate with the mesoderm undergoing segmentation to form the myotomes. In every case showing development, differentiation had progressed far beyond the stage of transplantation.

1. Early rat embryos (8 and 9 days) when transplanted upon the chorioallantoic membrane of the chick may undergo a definite differentiation of their tissues during the ensuing period of incubation. Heart, ear vesicles, liver, gut and supporting tissue may undergo their formation in such grafts.

2. Organization of tissue elements into definite morphological units has been secured. This shows that the organizing capacity is present even when the embryonic rudiments are developing under abnormal conditions of temperature, pressure, and with foreign nutrition through the blood supply of the chick.

5868

X-Ray Diagnosis of Ileus. Comparison of Results Obtained by Roentgenograms in Horizontal and Upright Positions.

ALTON OCHSNER.

From the Department of Surgery, School of Medicine, Tulane University of New Orleans.

The value of plain roentgenograms of the abdomen without the administration of contrast substances in the diagnosis of ileus has been repeatedly emphasized by clinicians. Goehl, Lynch, Borman, and Wangensteen[1] found experimentally that roentgenologic evidence was of less value in the presence of strangulation than in simple obstruction. The roentgen diagnosis of ileus is based upon the fact that in obstruction there is an accumulation of gas and fluid proximal to the point of obstruction which can be visualized roentgenologically. Whereas the presence of gas visualized in the roentgen plate is of diagnostic value, it is considered by many observers[2] that the finding of multiple fluid levels (demonstrated by obtaining the roentgenogram in such a way that the junction between gas above and fluid below may be visualized) is of greater diagnostic importance.

The present investigation was undertaken to compare the results obtained with roentgenograms taken in the horizontal position and those taken in the upright position, the latter technic demonstrating fluid level formation.

Ninety-four observations were made on 19 dogs. At each observation, 2 roentgenograms of the abdomen were obtained, one with the animal in the horizontal position, and the other in the upright position. The x-rays were made by means of a specially constructed radiographic animal board.[3]

A simple obstruction of the jejunum and terminal ileum was produced in 3 and 7 animals, respectively. A "strangulated" obstruction of the jejunum, ileum and sigmoid flexure of the colon was produced in 2, 5, and 2 animals, respectively. Roentgenograms were taken at intervals after the obstruction, varying from 1 to 75 hours.

In the high jejunal obstructions, little difference could be detected

[1] Goehl, R. O., Lynch, F. W., Borman, C., and Wangensteen, O. H., Proc. Soc. Exp. Biol. and Med., 1930, **27**, 952.

[2] Ochsner, Alton, and Granger, Amedee, *Ann. Surg.*, 1930, **92**, 947.

[3] Ochsner, Alton, and Gage, I. M., *Radiology*, in press.

in the films taken in the horizontal position between the simple and strangulated obstructions, but in the roentgenograms obtained with the animal in the upright position, there was considerable difference, in that 50% more fluid levels were present in films of animals with strangulated obstruction than in those with simple obstruction. The earliest appearance of gas accumulation or fluid levels, both in the simple or strangulated obstruction, was 3 hours after the obstruction.

In obstructions of the ileum, there was considerable difference between roentgenograms obtained from animals with strangulated and simple obstructions. The films of the animals with an associated strangulation showed evidence of gas accumulation and fluid level formation within one to 2 hours after the obstruction, whereas in the films of animals with simple obstruction, the earliest evidence of gas accumulation was 5 hours, which, however, did not become very marked until 7 to 9 hours after the obstruction. Similar results were obtained in strangulated obstruction of the sigmoid.

In comparing the roentgenograms taken in the horizontal position and those taken with the animal in the upright position, there was relatively little difference except that possibly gas alone without the evidence of fluid could be demonstrated earlier than gas and fluid. However, the findings of gas and fluid as evidenced by multiple fluid levels are so definite and pathognomonic, it is believed that when it can be demonstrated it is of much more diagnostic importance than gas alone.

5869

Experimental Obstructive Jaundice. Effect on Fibrinogen and Coagulation of the Blood.

WALTER MOSS. (Introduced by Alton Ochsner.)

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There has been very little work done to convince one that a deficiency of fibrinogen in the blood does or does not result from obstructive jaundice and cause an increase in the coagulation time. As fibrinogen plays such a prominent rôle in blood coagulation, it seems feasible that an experimental investigation should be made to determine the effect of obstructive jaundice on the amount of fibrinogen in the plasma and the subsequent effect on the time of coagulation of the blood.

Twenty-seven dogs were subjected to acute obstructive jaundice by ligating and dividing the bile ducts. Estimations of the fibrinogen content, icterus index, and coagulation time of the blood were made at intervals. Six of the animals died within 3 or 4 days after operation. Of the remaining 21, all died (average length of life 31.6 days), the only assignable cause of death being diffuse hepatitis. This was found in an overwhelming majority of the cases to be fatty necrosis with degeneration in the centers of the lobules.

Conclusions. Fibrinogen content of plasma is not decreased but actually increased in acute experimental obstructive jaundice, probably due to irritative effect of bile on liver. Coagulation time remained above normal limits, but did not parallel the fibrinogen content of the plasma. Coagulation time is not a satisfactory index of a hemorrhagic tendency in the presence of jaundice. Diminution of fibrinogen content does not parallel the degree of liver destruction. The Wu method for determination of fibrinogen is not satisfactory when exact quantitative analysis of blood proteins is done. The Tyrosine equivalent for dogs was found to vary from 12.5 to 19.7. This was learned after determinations were made from 24 samples of blood. For comparative analysis as herein used, the Wu method serves the desired purpose.

5870

Motility of Gastro-Intestinal Tract of Rats on Vitamin D Deficient Diet with Varied Minerals.

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From the Department of Medicine, Tulane University, and the Department of Pharmacology, Columbia University.

Previously we reported[1] that rats fed a deficient vitamin D diet with unbalanced minerals show a marked hypomotility of the gastro-intestinal tract. Subsequently[2] it was demonstrated that rats fed a diet deficient in vitamins A and D but with an adequate mineral balance show a gastro-intestinal motility similar to that of normal animals. The hypermotility produced by the absence of vitamin A[3] was apparently balanced by the hypermotility produced

[1] Menville, L. J., Blackberg, S. N., and Ané, J. N., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 758.

[2] Menville, L. J., Ané, J. N., and Blackberg, S. N., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 894.

[3] Gross, L., *J. Path. and Bact.*, 1924, **27**.

by the absence of vitamin D and for this reason it was believed that the unbalanced mineral content of the diet played a very small part in the hypomotility observed in the rats fed a diet deficient in vitamin D with unbalanced minerals. The present experiments were undertaken to substantiate or to disprove this hypothesis.

Three different groups of rats were used. One group was fed a diet deficient in vitamin D with unbalanced minerals for a period of 5 weeks. Another group was fed a diet deficient in vitamin D with an adequate mineral content for the same length of time. The third group was fed a normal diet for the same period. All the rats were made to fast and abstain from water for 24 hours when they were fed a 10 gm. mixture of 3 parts of buttermilk and one part of barium sulphate. Fluoroscopic examinations were made of all groups every 15 minutes.

A hypomotility of the gastro-intestinal tract was observed in the 2 groups of rats fed a deficient vitamin D diet. This observation was noted by us in rachitic rats. Our 2 groups of rats in this experiment would seem to indicate that the mineral content of the diet administered apparently played no part in the alteration of the gastro-intestinal tract.

TABLE I. (Averages)

Diets.	Cecum App. Time		Stom. Emp. Time		Sm. Int. Emp. Time		Colon Emp. Time
	hr.	min.	hr.	min.	hr.	min.	hr.
—D. High Ca, low P	1	55	8	27	9	56	93
—D. Normal minerals	1	56	7	56	9	16	96
Normal	2	15	6	18	7	46	60

Conclusion. It would appear from our observations that the cause of the hypomotility of the gastro-intestinal tract of rachitic rats is due to a lack of vitamin D and not to the unbalanced minerals of the diet.

5871

Some High Tremor Frequencies.

LEE EDWARD TRAVIS AND JOHN M. DORSEY.

From the Psychopathic Hospital, Iowa City, and the Psychopathic Hospital, Ann Arbor.

Hill[1] demonstrated that in addition to the usual tremor frequency

[1] Hill, A. V., *J. Physiol., Proc. Physiol. Soc.*, 1927, **55**, xiv.

of 8 to 12 tremors per second, a tremor frequency of around 50 tremors per second could be recorded. He found that the tremors of higher frequency and lesser excursion were superimposed upon those of lower frequency and greater excursion. Travis and Hunter[2] called attention to the striking similarity between records of tremors and those of action currents and later recorded tremors of a rate as high as 200 per second.[3] The present paper reports the recording of tremors of a rate as high as 500 per second.

For the recording of the tremors we used an electrical current generator to activate, with or without the aid of amplification, a super-sensitive element of a Westinghouse oscillograph. All tremors were recorded from the extended forefinger of the firmly supported hand. Several healthy individuals served as subjects. By means of an electrical filter and amplifying system we were able to by-pass and amplify greatly only tremors of frequencies above 130 per second. When such recordings are compared with those obtained without the use of filter and amplifier some interesting relationships are revealed (Fig. 1). It is to be noted that each large tremor (in

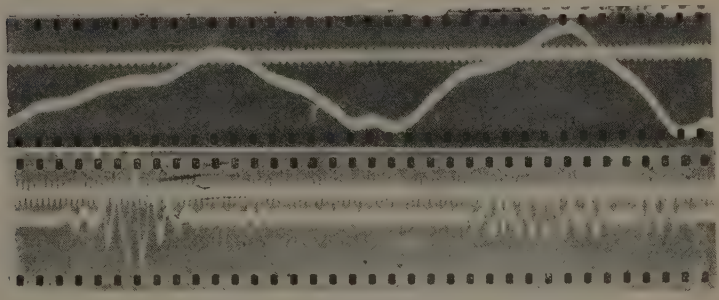

FIG. 1.

Records of tremors from the extended forefinger with the hand firmly supported. The upper record was obtained without the use of filter and amplifier. The lower record was obtained by by-passing and amplifying only tremors above 130 per second. Time is recorded in 0.002 sec.

upper record) enduring approximately 0.1 sec. is really composed of a group of several oscillations (in lower record) occurring at a rate of about 200 per second. Further the tremor line between the volleys is not quiet. It presents both smaller oscillations of the same frequency as the larger waves in the volleys, and other generally still smaller oscillations of a frequency as high as 500 per second.

[2] Travis, L. E., and Hunter, T. A., *Am. J. Physiol.*, 1927, **81**, 355.

[3] Travis, L. E., and Hunter, T. A., *J. Gen. Psychol.*, 1931, **5**, 255.

To be sure that all of the oscillations recorded come from the extended finger several control tests were made. With either a constant pressure or with no pressure on the platform of our generator no oscillations were detected. With either the input wires of the amplifier short circuited or with them open no disturbances of any kind appeared in the records. Great care had to be taken to eliminate the effects of tones and noises. However, we feel certain that mechanical, acoustical and electrical artifacts do not appear in our recordings.

There remains the problem of allocating the possible source of these tremor frequencies.

5872

Cultivation of the Virus of Common Cold and Its Inoculation in Human Subjects.

H. M. POWELL AND G. H. A. CLOWES.

From the Lilly Research Laboratories, Indianapolis.

In a series of papers Dochez[1] and his associates have presented evidence for the virus etiology of the common cold and have described experiments on the cultivation of this virus in tissue media and its inoculation into human subjects and chimpanzees. The purpose of this investigation has been to cultivate the virus of common cold and during the period of the year when the incidence of common colds is low to conduct human inoculation experiments on volunteers not subject to isolation.

The strain of common cold virus employed in these experiments was derived from a patient, "A. R.," suffering from a cold of more than usual severity. Nasal washings were obtained on March 26th, 1931, within the first 24 hours of the onset of the cold. Ten cc. of Tyrode solution were flushed through each nostril and expelled through the mouth. These washings were promptly passed through a Seitz filter and cysteine hydrochloride added to a concentration of 1:2000. The filtered washings were introduced in 1 cc. amounts

[1] Dochez, A. R., Shibley, G. S., and Mills, K. C., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 562; **27**, 59; *J. Exp. Med.*, 1930, **52**, 701. Shibley, G. S., Mills, K. C., and Dochez, A. R., *J. Am. Med. Assn.*, 1930, **95**, 1553. Dochez, A. R., Mills, K. C., and Kneeland, Y., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 513; **29**, 64.

into 9 cc. hashed chick embryo tissue culture medium containing cysteine hydrochloride in a concentration of 1:2000. The culture was incubated under vaseline seal at 37°C. for 4 days. A Seitz filtrate was prepared from this first culture generation and stored under vaseline seal at 4°C. Further tissue cultures and filtrates were prepared in series as described above, the periods of incubation being from 4 to 5 days and the intermediate periods of ice-box storage of the Seitz filtrate averaging 5 to 6 days. At the present time this cold virus is in the 31st generation and has been cultivated successfully outside the body for a period of 8 months.

Attempts to induce common colds by inoculation into human volunteer subjects were carried out as follows:

About 1 cc. of virus filtrate was instilled into each nostril as the subject lay upon his back. The head was turned toward one side and then toward the other. After a few minutes the instillation was repeated and the subject turned over upon his face to allow of more adequate wetting of the membranes. This procedure was usually carried out in the morning and repeated about 5 or 6 hours later in a routine fashion.

These test subjects were not isolated but were selected from large groups of comparable individuals working under the same conditions in a large manufacturing unit. The experiments were conducted during the spring and summer when the incidence of colds was low in the group of workers and their families from whom the test individuals were selected. Only those individuals were subjected to test who had not suffered from colds within 2 months of the date of the experiment but were known to average at least 2 or 3 colds each winter, which would indicate that they were susceptible to colds. Cultures were made for 2 days prior to inoculation and all individuals showing hemolytic streptococci and pneumococci of fixed types were excluded from test.

Before and during each such inoculation experiment the incidence of common colds was ascertained in the control group consisting of individuals working in the same environment and under the same conditions as those subjected to the test. Transmission experiments were not started at any time when the incidence of colds in the control group proved to be more than 10% during the week preceding the experiment.

Table I gives a record of the cultivation of this strain of common cold virus and the results of inoculation experiments on presumably susceptible human test subjects. It will be seen that from 60 to 80% of the subjects used experimentally contracted these in-

TABLE I.
"A.R." Strain of Common Cold Virus: Human Inoculations.

Cold Virus Generation	Human Inoculation tests	Inoculated Groups			Control Groups		
		No. Subjects Used	No. Subjects Contracting Colds	% Contracting Colds	No. of Individuals	No. Exhibiting Colds	% Exhibiting Colds
2	4/7/31	3	2	66			
4	4/23/31	5*	3	60			
6	5/8/31	5	4	80	717	71	10
11	6/8/31	6	4	66	700	56	8
13	6/16/31	4	3	75	496	50	10
18	7/21/31	5	3	60	210	16	8
27	11/11/31	4*	3	75	200	10	5
Total		32	22	69%	2323	203	8¾%

* In each of these instances the person conducting the intranasal inoculation experiments was exposed to the cold virus being used, and in each case contracted a head cold at the same time as the positive test subjects. The number of subjects used in either case, however, does not include the operator.

oculation colds. Of the 22 individuals who developed colds as a result of inoculation 19 had moderately severe to severe head colds, running the usual course without complications. Two individuals had colds of comparatively short duration and 1 developed a mild bronchitis which was treated medically with uneventful recovery. While the incidence of colds in the inoculated cases was 69%, the incidence of colds in 2323 control individuals was less than 9%.

The length of time which elapsed between the preparation of the Seitz filtrate and its inoculation into test subjects was less than a day in the 6th, 13th, and 18th generations, was 2 days in the 4th generation, 3 in the 11th generation, 4 in the 2nd generation, and 8 in the 27th generation. It is particularly interesting to note that at the 27th generation, 7 months after the isolation of the cold virus strain, the incidence of infection was 75% and the resulting colds of more than usual severity. Since the 27th generation gave a high incidence of colds of unusual severity it is obvious that the virus has remained unimpaired in the Seitz filtrate at ice-box temperatures for a period of at least 8 days.

Since the dilution of the filtrate was 1 to 10 for each generation, the total dilution of the original virus culture at the 27th generation was 1 to 10^{27}. The maintenance of this culture at full virulence for 27 generations and so long a period as 7 months fully confirms the conclusion reached by Dochez that the virus of the common cold may be readily cultivated in an appropriately prepared chick embryo medium and readily transmitted to suitable human test subjects.

Immune phenomena observed in the course of these experiments will be made the subject of a future communication.

5873

Salt as a Factor in Edema Formation Following Plasmaphoresis.*

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It is well known to clinicians that it is sometimes possible to rid a patient with nephrosis or with nephritis of visible edema by the deprivation of salt. When even moderate quantities of salt are ingested, visible edema may appear in 24 to 48 hours. There is a close correlation between edema formation and reduction of the colloid osmotic pressure in these cases consequent upon loss of protein from the blood plasma.

Leiter,[1] and Barker and Kirk[2] have bled dogs, thrown away the plasma and returned the cells suspended in Ringer's solution to the vascular system. Massive edema was obtained when the protein content of the blood was reduced. They obtained massive edema at protein values frequently higher than we have observed in our patients with edema. We believe this was because they gave large quantities of salt in physiological salt solution each day throughout their experiment. They gave 1500 cc. of physiological salt solution by mouth each day, equalling 13.5 gm. of NaCl per day, equivalent to about 60 gm. a day in an adult.

We have bled 7 dogs (500 cc. or more 3 times a day) removed the plasma which was replaced by Ringer's solution and returned the corpuscles to right heart. Only a very slight edema of the gluteal folds and the skin of the extremities was obtained when the total protein content of plasma was below 3% and osmotic pressure of the plasma colloids below 10 mm. Hg. The dogs were given 1500 cc. of tap water each day and the protein content held at close to the above figures. The dogs put out nearly as much water each day as was ingested. On giving 1500 cc. of 0.9% salt solution the output of urine immediately fell far below the intake and mas-

* Carried out with the aid of a grant from the Emma Sachs Plotz Fund.

[1] Leiter, L., *Arch. Int. Med.*, 1931, **48**, 1.

[2] Barker, M., and Kirk, E. J., *Arch. Int. Med.*, 1930, **45**, 319.

sive edema and ascites appeared, showing that NaCl is a factor in producing edema where the colloid osmotic pressure reduction leads to "Oedembereitschaft" or Edema Tendency.

The exact mechanism whereby the NaCl leads to increased edema formation we are leaving for a future publication. Suffice it to say that these experiments prove that the statement that salt is held back through renal insufficiency in nephroses and nephritis with edema is probably false.

We also noted in our work that the blood corpuscles should not be returned to the vascular system by intravenous injections because this slight injury to the veins tends to cause increase in venous pressure and local edema when "Oedembereitschaft" is present. The experiment is clean cut only when the corpuscles are returned to the right ventricle.

Conclusions. Lowering plasma proteins to below 3% and osmotic pressure of plasma colloids to below 10 mm. produces a tendency to edema formation. The edema is very slight and does not increase rapidly until NaCl is given, when the urinary output falls suddenly and massive edema appears.

5874

Colloidal Gold Test for Serum Antibodies in Poliomyelitis.*

FREDERICK EBERSON AND WILLIAM G. MOSSMAN.

From the Clinical and Research Laboratories, Mount Zion Hospital, San Francisco, California.

In a previous communication[1] a method was described for the detection and titration of immune bodies in poliomyelitis, with reference to the applicability of the test in the study of susceptible persons, carriers, therapeutic value of serums from normal adults, and in relation to prognosis and convalescence.

A total of 363 serums has been studied to date. These included 100 normal adults and 100 normal children (age groups 6 weeks to 19 years), 58 convalescent serums (monkey, adult human, and children), 76 normal animals (monkey, horse, goat, and sheep),

* Work done under a grant for poliomyelitis research supported by anonymous donations. Read before Section N (Medical Sciences) on June 16, 1931, at the 88th meeting of the American Association for the Advancement of Science, held at Pasadena, California, June 15-20, 1931.

[1] Eberson, F., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 405.

and 29 immune animal serums (monkey, horse, goat and sheep).

The results have agreed with known facts. The specificity of the test has been demonstrated also by observations made during all stages of poliomyelitis in the human and in the monkey.

The progressive development of immune bodies could be shown to occur during convalescence, particularly in those cases where rapid and complete recovery ultimately took place. These antibodies could not be found in the blood serum of patients or animals with a poor outlook as to recovery. When the disease went on to a protracted convalescence with poor or slow restoration of muscular activity, these immune bodies were found to be weak or negligible in quantity.

The serums from infants and very young children did not show any protective properties whatsoever and the same was true for 34% of adults, thus agreeing with the known facts of susceptibility to poliomyelitis and the neutralizing power against the virus of serums from certain normal adult persons. The following results were obtained in 200 serums that were tested for poliomyelitic antibodies: 100 *adults,* ranging in age from 25 to 50 years, gave a *positive* test for immune bodies in 64% of the cases (30%, strongly positive; 20%, moderately strong; and 14%, weakly positive). One hundred *children,* ranging in age from 6 weeks to 19 years, gave a *negative* test in 77% of the cases. Of 50 in the *age group from under 1 year to 8 years,* 90% gave *negative* tests for immune bodies, 6% a weakly positive, and 4% a moderately strong test; among 50 in the *age group of 9 to 19 years,* 64% were *negative* and 36% were positive for immune bodies (12%, strongly positive; 16%, moderately strong; and 8%, weakly positive). (Table I.)

The nature of the precipitation phenomenon exhibited by certain serums subjected to the colloidal gold test was studied more minutely by means of *in vivo* neutralization experiments in the monkey. It was especially desirable to evaluate the protective property of serum obtained from normal adults. For this purpose 2 serums were selected from such a group, one serum giving a negative and the other a definite positive result with the colloidal gold test. In the experiments, a highly potent virus obtained through the courtesy of Dr. Simon Flexner was used. The virus filtrate in 0.5 cc. and 0.62 cc. amounts from a 10% and 20% emulsion was mixed with 0.5 cc. amounts of serum (previously inactivated one-half hour at 56°C), incubated at 37°C for one hour, allowed to stand over night

TABLE I.

Incidence of Positive and Negative Tests for Antibodies in Different Age Groups.

Adults (Age 25-50)

Number	Negative	Positive			Total Positive
		Weak	Moderately Strong	Strong	
100	36	14	20	30	64

Children (Age 6 Weeks-19 Years)

Age	Number	Negative	Positive							Total Positive	%
			%	Weak	%	Moderately Strong	%	Strong	%		
Under 1 yr.	4	4	100.00	0	0	0	0	0	0	0	0
1-4 years	19	19	100.00	0	0	0	0	0	0	0	0
5-8 years	27	22	81.5	3	11.1	2	7.4	0	0	5	18.5
Total	50	45	90.0	3	6.0	2	4.0	0	0	5	10.0
9-14 years	35	25	71.4	2	5.7	5	14.3	3	8.6	10	28.6
15-19 years	15	7	46.6	2	13.3	3	20.0	3	20.0	8	53.3
Total	50	32	64.0	4	8.0	8	16.0	6	12.0	18	36.0
Total	100	77	77.0	7	7.0	10	10.0	6	6.0	23	23.0

in the icebox (4°-6°C), and the entire contents injected intracerebrally under ether anesthesia and careful surgical technic. Six monkeys were employed; 2 received normal, unpreserved human serum giving a negative test (7777777777), 2 others a human serum with a positive test (1111224477), and the other 2 received the virus alone. The control animals became paralyzed on the sixth to the eighth day (one died on the seventh day), the animals that

received the strictly normal serum came down on the seventh to the ninth day with typical poliomyelitis, and the 2 animals receiving the positive test serum remained free of all symptoms. In these instances the serum was effective against approximately 50-60 M.L.D. as calculated on the usual activity of the virus, and the specificity of the *in vitro* colloidal gold test was thus demonstrated. These experiments have a direct bearing upon the possibility of employing such "neutralized" or "protected" mixtures of virus and serum as determined by our rapid test for the purpose of active immunization. Such studies, now in progress on a larger scale, aim to use an appreciable excess of immune serum in combination with virus in such amounts that the colloidal gold test will still give a positive reaction with the "protected" mixture.

The specificity of the test was further shown by experiments in which serums were studied in the following manner: (1) Different concentrations of virus in fixed quantities were combined with varying dilutions of serum. (2) Different dilutions of a 5% or 10% virus in fixed amounts were combined with fixed quantities of serum. The protocols which will appear in the complete report may be summarized as follows: The precipitation of the colloidal gold preparation by immune serums did not occur in the presence of poliomyelitis virus when the virus was present in excess. Similarly, in the presence of an excess of antibodies due to incomplete absorption on the part of a given amount of virus, the serum gave a positive precipitation test. With encephalitis and herpes viruses no absorption occurred and immune serums gave typical positive precipitation reactions.

The application of the colloidal gold test in the study of poliomyelitis has been shown to have a direct bearing and practical value in the problems concerning: (1) Selection of donors' serum for therapeutic use in poliomyelitis. (2) Study of susceptibility to this disease among the general population. (3) Evaluation of therapeutic potency of serums from human and animal sources. (4) Prognosis during the course of poliomyelitis as related to the progressive development or complete absence of serum antibodies. (5) *In vitro* selection of "protected" mixtures of poliomyelitis immune serum combined with virus for purposes of active immunization.

5875

Anthelmintic Properties of Certain Alkyl Cresols.*

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In a study of anthelmintics being carried out in this laboratory, it has been shown that certain resorcinols have marked anthelmintic properties,[1] hexylresorcinol having the most intense action of the normal alkyl resorcinols. Both hexylresorcinol and heptylresorcinol have been given in ascaris, hookworm, and trichuris infestations, hexylresorcinol being found to have the greatest efficiency.[2] The latter has now been given by us to over 2,000 cases controlled by the Stoll egg counting method, without any indication of toxic effects, and with an average efficiency of approximately 90% in ascaris, 85% in hookworm disease, and 55% in trichuriasis.[1, 3] The lower resorcinols were found to be less active *in vitro,* resorcinol itself having relatively very little action. It was first shown by Johnson and Lane that the antiseptic action of resorcinol could be increased by the introduction of alkyl groups and that this antiseptic action increased with the length of the straight alkyl chain up through butyl resorcinol.[4] Leonard then showed that the antiseptic action of these alkyl resorcinols reached a peak with hexylresorcinol and that this member of the series had the least toxicity.[5] This idea of introducing alkyl groups into substances with known antiseptic action was recently taken up by Coulthard, Marshall and Pyman, who showed the variation of phenol coefficients in homologous series of phenols.[6] As with the resorcinols a similar increase in the phenol coefficient with an increase in molecular weight of the alkyl chain, the maximum being found in 5-n-amyl-o-cresol, was demonstrated.

* The funds for carrying out this work were given by the International Health Division of the Rockefeller Foundation.

[1] Lamson, P. D., Caldwell, E. L., Brown, H. W., and Ward, C. B., *Am. J. Hyg.*, 1931, **13**, 568.

[2] Lamson, P. D., Caldwell, E. L., Brown, H. W., and Ward, C. B., *Am. J. Hyg.*, in press.

[3] Lamson, P. D., Brown, H. W., Robbins, B. H., and Ward, C. B., *Am. J. Hyg.*, 1931, **8**, 803.

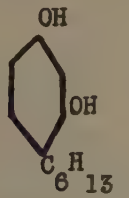
[4] Johnson, T. B., and Lane, F. W., *J. Am. Chem. Soc.*, 1921, **43**, 348.

[5] Leonard, Veader, *J. Am. Med. Assn.*, 1924, **83**, 2005.

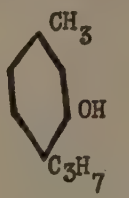
[6] Coulthard, C. E., Marshall, J., and Pyman, F. L., *J. Chem. Soc.*, 1930, 280.

The fact that thymol, a very effective anthelmintic which is 4-iso-propyl-m-cresol is infinitely more effective on ascaris than simple m-cresol suggested to us that straight chain cresols might be made with still greater anthelmintic properties.

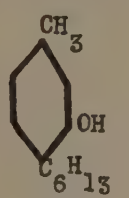
As we could not obtain these compounds we have as yet been unable to make a comparison of the anthelmintic properties of all of them. However hexyl and heptyl m-cresols were chosen for investi-



Hexylresorcinol



4-iso-propyl m-cresol (Thymol)



4-n-hexyl m-cresol

gation, synthesized in this laboratory, and compared with the hexyl- and heptyl-resorcinols already studied by us. These substances were found to be very active on ascaris *in vitro* although slightly less so than hexylresorcinol and effectively removed ascaris from dogs. This is of interest in showing an increase in anthelmintic properties with the increase in length of the alkyl chain, as compared with the relatively ineffective phenol or m-cresol. The introduction of the methyl group into phenol is well known to reduce its toxicity and it might be expected that these higher cresols would show a still less toxic action. Our experiments with 4-n-hexyl-m-cresol in rats and dogs confirm this view, and a paper by Broom[7] which has just appeared, since this note was sent to press, shows the 4-n-amyl-m-cresol to have a toxicity less than that of hexylresorcinol in animals, and this substance has been taken in therapeutic amounts by man without any indication of intoxication.[8] Because of certain properties of 4-n-hexyl-m-cresol, namely its being a liquid, and causing less local irritation than hexylresorcinol, further experiments in both animal and man are being carried out in order to determine its possible value as a human anthelmintic.

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[7] Broom, W. A., *Brit. J. Exp. Path.*, 1931, **12**, 327.

[8] Coulthard, C. E., *Brit. J. Exp. Path.*, 1931, **12**, 331.

5876

Can Protein Act as a Carrier in Penetration?

MARIAN IRWIN.

From the Laboratories of the Rockefeller Institute for Medical Research.

1. Experiments[1] were carried out at 10°C.

2. Gelatin was placed in cresyl blue solution at pH 9 and at pH 5.5. Dye solutions were changed before alteration in the dye occurred. After 24 hours gelatin was removed, washed in distilled water, and melted in a test-tube. The concentration of the dye in the gelatin was determined colorimetrically. More dye was taken up by gelatin at pH 9 than at pH 5.5 due to greater concentration of negatively charged protein ions at pH 9 capable of combining with the dye cations.

3. The gelatin stained at pH 9 was placed in buffer solutions at pH 9 and at pH 5.5. After 12 hours the concentrations of the dye in the buffer solutions were determined colorimetrically. More dye passed out of the gelatin into the buffer solution at pH 5.5 than at pH 9. This is due to the decrease in the negatively charged protein ions at pH 5.5.

4. Experiments were repeated 10 times and without exception the above results were obtained.

5. These results are significant as indicating the following possibility. If the dye penetrates a living cell in a dissociated form from cresyl blue solution we may picture the mechanism as above described when the external protoplasmic surface and the vacuolar surface consist of protein or a substance behaving like it. No matter how readily the dye is taken up by the surface, it cannot penetrate into the interior of the cell unless it is capable of being given up at the inner side in such a manner as described above. If both the acid and basic dyes penetrate we may assume that these surfaces consist of a mixture of proteins with various isoelectric points.

[1] Loeb, J., Proteins and the theory of colloidal behavior, McGraw-Hill Book Co., Inc., New York, 2nd ed., 1924.

5877

The Therapeutic Value of Pneumococcus Type VII (Cooper) Serum.*

JESSE G. M. BULLOWA.

From the Littauer Pneumonia Research Fund of New York University and the Medical Service, Harlem Hospital.

Georgia Cooper[1] and her coworkers, working with cultures from pneumonia patients at Harlem Hospital and Bellevue Hospital, segregated Type VII from the miscellaneous group. During the past 3 years 121 patients invaded with Type VII pneumococcus have been observed at Harlem Hospital. During this period 1407 patients were admitted to the adult Pneumonia Series. The incidence of Type VII was 7.5%. The mortality among 85 cases treated without serum was 25.9%.

Serum has been employed on 17 adult patients and 2 children. It was administered for the most part until agglutinins were demonstrable by the whole blood stained slide technique. The cases were chosen when serum was available by the chance of their alternate admission to the hospital. One patient, to whom serum was administered on what was thought to be the 23rd or 24th day of her disease, came in on the 20th day of her illness, with the history of a chill and pain in the side at the onset. She was obese, had an aortic insufficiency, and had had dypsnea on effort for 2 years. She died of exhaustion. All the other patients recovered. One of those who received serum and recovered, suffered from a bacteremia which was apparently increasing prior to serum administration.

The cases are insufficient in number to establish the curative value, but they furnish suggestive evidence that adequate doses of Type VII antipneumococcic serum may have therapeutic value in patients suffering from Type VII pneumococcus pneumonia. Observation of the cases led me to believe that it was beneficial.

The lots of serum, the method of production and refinement and reactions are shown in Table I:

* Through the generosity of the Littauer Pneumonia Research Fund of New York University, and the Altman Foundation, serum was prepared under the direction of Dr. William H. Park at the Municipal Serum Farm, Otisville, N. Y., and was refined in New York by Banzhaf, and in Boston by Felton. Other serum was generously supplied by the Lederle Laboratories, Inc., where it had been produced under the direction of Mr. Stanley D. Beard, and refined by Mr. Joseph H. Greene.

[1] Cooper, *J. Exp. Med.*, 1929, **49**, 461.

TABLE I.

No.	Preparation Made by	Units per cc.	Intravenous Doses	Patients	Chills	Other Reactions
1	Dept. of Health Prep. 937-1-A	Type 7 800 8 80	5	2	1 (slight)	
2	Dept. of Health Prep. 2	7 800 8 80	2	2	2 (severe)	
3	Dept. of Health Prep. 3	7 800± 8 80	11	3	4 (moder.)	
4	Dept. of Health Prep. 5, Lot 708	7 800± 8 80	9	1	0	4 doses caused sweating
5	Dept. of Health Serum refined by Felton. Polyvalent I and II	7 800 8 80	45	9	0	Dyspnea relieved with adrenalin
6	Dept. of Health Prep. 936-1-B		9	4	8 (moder.)	
7	Lederle 21-H-2	4 150 5 1000 7 750 8 500	10	3	0	One dose: cyanosis, dyspnea.
8	Lederle 21-H-6	4 300 5 750 7 1500 8 500	1 (Same patient given 21-H-12 without reaction)	1	1 (moder.)	Very severe cyanosis, sweating and dyspnea
9	Lederle 21-H-8	4 750 5 500 7 2000 8 500	28 (Doses as large as 40 cc.)	4	0	Two doses: cyanosis and dizziness
10	Lederle 21-H-12	4 250 5 750 7 5000 8 500	4	3	0	One dose caused dyspnea and patient complained of feeling upset

Method of Refinement was as follows:

No. 1. 8 mos. immunization of horse 184. Diluted one-half saturation with 33 1/3 $(NH_4)_2SO_4$. Filtered. Ppt. washed and saturated with NaCl. Filtrate washed and brought up to 52% saturation with $(NH_4)_2SO_4$. Dialyzed 4 days.

No. 2. Horse 291. 7 mos. on 5 types. 8 mos. on 2 types. Heat killed antigen. Plasma dialyzed and then diluted with 3 volumes of H_2O. pH changed to 5.1. Antibody precipitated at pH 5.9.

No. 3. Prepared same as Prep. 2 except antibody precipitated at pH 6.8.

No. 4. Horses 282-241. Heat killed antigen. Serum dialyzed. pH changed to 5.1 and acid ppt. destroyed. Antibody precipitated at pH 6.8.

No. 5. Serum from same horses as No. 4. Sod. sulphate prep. Elimination of acid fraction and the total water insoluble protein.

No. 6. Diluted one-half 50 cc. N/HCl. Saturated to 6% with saturation $(NH_4)_2SO_4$ and saturated NaCl. Filtrate brought to 33% saturation. Filtered and dialyzed.

No. 7. Formolized antigen. Immunization 12 mos. Serum diluted with H_2O and ppt. with 45% $(NH_4)_2SO_4$. Dialyzed. Ppt. acidified. Acid soluble fraction precipitated and dissolved with 1.5% NaCl.

No. 8. Chilled to 1.5°C. Ppt. with 20% alc. Filtered at 5°C. Ppt. dissolved. Acid soluble fraction dissolved and diluted with distilled water.

No. 9. Re-refined 21-H-6 (No. 8).

No. 10. Formolized antigen, 15% alc. Chilled. Diluted and acidified. Acid soluble fraction neutralized and diluted. Cloudy—therefore re-refined.

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Occurrence of Fatty Livers in Rats Fed a Diet Containing Dried, Whole Liver.

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Our observation of the presence of an abnormal amount of fat in the livers of rats fed a diet high in protein was first made in a series of rats in which we were attempting to determine the stages of development of chronic nephritis. In rats placed on Liver Diet I and killed from 2 to 3 months later, we found that we could tell from the presence of fatty infiltration of the liver which rats were on the diet and which were not; whereas, we were unable to detect any difference between the kidneys of the rats on the Control or the Liver I diet. This observation led to a search for the cause of the phenomenal difference in the livers of the rats fed the 2 diets.

The livers of rats fed Liver Diet I contain very large amounts of cholesterol and fat but less than the normal amount of lecithin. The

TABLE I.
Fat Content of Rat Livers.

Rat No.	Days on Diet	Cholesterol	Lecithin	Fatty Acids less Lecithin	Diet	I	IV	VII
		%	%	%				
202	59	2.67	2.37	9.48	Beef liver, dried	75		
203	59	2.77	2.27	10.32	Liver residue, aq. ext.		75	
204	59	3.66	2.25	10.51	" " alc. "			63
205	64	3.20	2.21	11.48	Lard	16	15	28
206	64	2.94	2.11	10.97	Yeast, dried	5	5	5
207	64	2.14	2.31	10.32	Cod liver oil	3	3	3
					Salt mixture*	1	1	1
212	61	0.49	3.24	3.34	Calc. carb.		1	
213	61	0.47	2.73	3.26				
214	61	0.36	2.82	2.70				
215	62	0.33	2.42	1.97				
216	62	0.45	2.63	3.39				
217	62	0.51	2.98	3.08				
224	58	0.44	3.01	3.36				
225	58	0.31	3.12	3.17				
226	58	0.39	3.20	3.49				
227	62	0.46	3.11	3.98				
228	62	0.68	3.13	5.44				
229	62	0.29	3.04	3.96				

* Salt mixture, Osborne and Mendel.

values for cholesterol have ranged from 2.14 to 5.51% and those for total fatty acids minus lecithin from 9.48 to 11.48%. The values for lecithin have varied from 2.11 to 2.37%.

When the liver residue remaining after the aqueous extraction of the pernicious anemia fraction replaces the dried, whole liver in the above diet, there is no excessive deposition of fat in the liver, (Liver Diet IV). Likewise, when dried, whole liver extracted with hot 95% alcohol is fed, fatty livers are not found, (Liver Diet VII).

All of these diets are high in fat and protein and contain very small amounts of carbohydrate. Liver Diets I and IV contain about the same large quantities of cholesterol and lecithin, while Liver Diet VII is practically free of these substances. The results with Liver Diet IV show that the development of the fatty livers is not caused by the large amounts of protein and fat in these diets.

Neither the addition of 1% of uric acid nor of 5% of yeast nucleic acid to Liver Diet IV gives rise to the typical fatty condition which results from the feeding of dried, whole liver.

All of the rats on Liver Diet I showed marked, diffuse fatty infiltration of the small and large droplet type by the end of 2 months. Aside from the abnormal amount of fat nothing of note was observed. The most marked accumulation of fat occurred in the liver cells in the peripheral part of the liver lobule. Rats fed Liver Diet IV showed no fat in the liver 2 months after they had been placed on the diet. The only thing worth noting in these livers was the apparent increase in the number of nuclei. Many of the liver cells showed 2 or 3 nuclei. The rats which ate Liver Diet VII showed a great irregularity in the amount of fat present. Rat 228 showed almost as much fatty infiltration as did some of the animals on Liver Diet I. Rat 225 showed no fat in the liver. The remainder of the rats in this series showed scattered single or small groups of cells filled with fat. The livers in this series showed nothing of special note aside from the variation in fat content.

From the pathological examination of these livers we determined only the presence of free fat as demonstrable by staining frozen sections with Scharlach R. The chemical determination of the fat, in terms of fatty acids, is the more accurate index of the total fat content of the liver.

It appears that there is a substance in dried, whole liver the ingestion of which causes the development of fatty livers. This substance is soluble in water and in 95% alcohol. Experiments are under way to determine whether this substance is present in fresh liver or whether it is formed during the drying process.